The Synthesis of Ribonucleic Acid *in vivo* in the Nuclei of Rat Brain Fractionated by Zonal Centrifugation

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1. Radioactive orotic acid, uridine and adenosine were administered to rats by intracisternal injection. The effects of the size of the dose, the specific radioactivity and time on the incorporation into the RNA of unfractionated nuclei of brain tissue were examined to establish appropriate conditions for studies of the relative activities *in vivo* of the various sorts of brain nuclei fractionated by zonal centrifugation. Uridine is incorporated more efficiently than either orotic acid or adenosine. 2. With [³H]uridine as precursor the astrocytes in zone (II) contain the highest radioactivity except at the beginning of the experiment when the neuronal nuclei of zone (I) are more highly labelled. This fraction utilizes [¹⁴C]orotic acid more readily than the nuclei of zone (II). The astrocytic nuclei of zone (III) show a general resemblance to those of zone (II). Considerable differences between the incorporations into the two types of oligodendrocyte nuclei in zones (IV) and (V) are observed. 3. The relative synthetic activities of the major types of brain nuclei *in vitro* and *in vivo* are discussed.

The nuclei of cells from adult rat brains have been fractionated by zonal centrifugation into five main classes which can be distinguished on a morphological basis (Austoker *et al.*, 1972). Zone (I) contained neuronal nuclei (70%) and astrocytic nuclei (23%). Zone (II) contained astrocytic nuclei (81%) and neuronal nuclei (15%). Zone (III) contained astrocytic nuclei (84%) and oligodendrocytic nuclei (15%), zone (IV) contained oligodendrocytic nuclei (92%) and zone (V) was virtually pure oligodendrocytic nuclei. The classes of nuclei showed significant differences in the amount of RNA and protein per nucleus and in the synthesis of RNA *in vitro*. The activity of RNA polymerase was highest in zone (II) followed by zone (I). The nuclei of zones (IV) and (V) had comparable polymerase activity, approx. 40% that of zone (II), and zone (III) had an activity intermediate between that in zone (I) and in zones (IV) and (V).

It is necessary to determine the extent to which assays of RNA polymerase activity reflect the rates of synthesis of RNA *in vivo*. Flangas & Bowman (1970) studied the metabolism of RNA *in vivo* for a period of 0.5–16 h after an intracisternal injection of [³H]cytidine or [³H]orotic acid and found that neurons incorporated more precursors into RNA than the glial cells of the rat brain. Løvtrup-Rein (1970), in experiments which involved repeated injections of [³H]adenine and [³H]cytidine at 30 min intervals up to 4 h into the fourth ventricle of the brain, found that the rate of incorporation of label into nuclear RNA was four times higher in astrocytes and neuronal nuclei than the other glial nuclei. Volpe

& Giuditta (1967), by using fractions enriched with either neurons or glial cells, found that the specific radioactivity in neuronal RNA was 2–3 times that in glial RNA between 3 and 6 h after administration of the label. Similar labelling patterns were found by Jarlstedt & Hamberger (1971), who incubated rabbit cortex slices in a medium containing [³H]uridine for various times, and after separating neurons and glia on a Ficoll gradient, found that the incorporations of radioactivity into RNA were similar after 30 and 60 min, but were 3 to 4 times higher into neuronal RNA after 3 h. These studies *in vivo* provided a general indication that the higher rate of RNA synthesis shown *in vitro* by neuronal and astrocyte nuclei (Austoker *et al.*, 1972) correlated with RNA synthesis in the intact animal. In this paper we report a detailed survey of RNA synthesis *in vivo* in the five classes of nuclei that we have isolated.

Experimental

Injection of radioactive tracers

Male albino rats (approx. 90 g) were injected intracisternally with [5-³H]uridine (27 Ci/mmol), [6-¹⁴C]orotic acid (60.8 mCi/mmol) or [8-¹⁴C]adenosine (52 mCi/mmol) (The Radiochemical Centre, Amersham, Bucks., U.K.). The quantities of radioactivity used are given in the Results and Discussion section. The apparatus used for injection was designed by Dr. F. Gonzalez-Mujica (personal communication).

A wedge-shaped perspex block and stand was constructed so that the nose of the animal, when held in position, is at 45° to the groove in the manoeuvrable

perspex wedge through which the injection needle passes. The animals were lightly anaesthetized with ether, placed on the stand and the block positioned against the skull. A probe with a very sharp point was passed along the groove in the perspex and used to drill a small hole in the skull, midway between the eyes. The radioactive precursor in 30 μ l of 0.9% saline, with a layer of 10 μ l of 0.9% saline above (to ensure that any droplets escaping after injection contained a minimum of radioactivity) was administered with a 100 μ l syringe (Scientific Glass Engineering, Melbourne, Australia). The needle was inserted through the small hole in the skull to a depth of 13 mm. At this depth the needle almost reaches the base of the skull and the liquid is injected intracisternally. To ensure that the radioactive precursor was being injected intracisternally, numerous experiments using blue dye were carried out to perfect the technique. In double-label experiments, [5-³H]uridine was always present. The time of incorporation before the animals were killed is given in the Results and Discussion section.

Preparation, fractionation and analysis of the nuclei

The animals were killed and the nuclei prepared and fractionated by zonal centrifugation as previously described (Austoker *et al.*, 1972). In experiments on unfractionated nuclei, the nuclei from the brains of each animal were separately isolated and processed. The nuclei from each brain (approx. 120×10^6) were resuspended in 1 ml of 0.32 M-sucrose–1 mM-MgCl₂, pH 6.4; and 400 μ l of this was used for determinations of radioactivity, which were carried out in duplicate. The number of nuclei was determined using a Coulter counter. In experiments on fractionated nuclei, the brains from 11 animals, similarly injected, were pooled and the nuclei isolated and fractionated. From 28 to 35 ml of the pooled diluted zonal fractions were used and the nuclei recovered by centrifugation at 1400 g for 60 min. This was carried out in triplicate for each zonal peak. The number of nuclei in each tube was determined by DNA analysis of nuclei recovered from a sample treated in a similar manner.

For determination of the radioactivity both in the nuclei recovered from zonal centrifugation and in the unfractionated nuclei, the nuclei were washed twice with 5 ml portions of 5% (w/v) trichloroacetic acid and once with 5 ml ethanol–chloroform (3:1, v/v). The final precipitate was dried, digested for 25 min in 0.2 ml formic acid and the radioactivity determined as previously described (Austoker *et al.*, 1972).

Results and Discussion

Studies with unfractionated nuclei

Before commencing a study of the RNA synthesized in the different classes of nuclei *in vivo*, it

was necessary to establish optimum conditions for the incorporation by preliminary experimentation on unfractionated rat brain nuclei. Parameters such as use of different radioactive precursors, dose size, time of incorporation and specific radioactivities of the radioactive precursors were investigated. The information derived from these studies guided the choice of conditions for studies with fractionated nuclei. In all cases an intracisternal injection was used to administer the radioactive precursors. If the animal was held firmly in the position described in the Experimental section, little difficulty was experienced with the injections.

The method was perfected using blue dyes to ensure that the liquid was injected intracisternally. All experiments on unfractionated nuclei were conducted on single animals, so that should the injection not be correct, any discrepancy was apparent immediately and the results were rejected. The animals recovered within 1–20 min after injection and showed no ill effects.

In experiments to determine the effect of radioactive dose on the extent of incorporation, it was shown that the radioactivity in the isolated nuclei after a given time was approximately linear with the size of the dose (Table 1). In most experiments 3 μ Ci of [¹⁴C]orotic acid and 3 μ Ci of [¹⁴C]adenosine were used, while 5 μ Ci of [³H]uridine was used in single-label experiments and 10 μ Ci in double-label experiments. In experiments to determine the effect of various specific radioactivities of the injected [³H]-uridine, without alteration of the amount of radioactivity that was supplied, it was found that a 100-fold decrease in specific radioactivity (from 27 Ci/mmol to 0.27 Ci/mmol) did not result in a change of radioactivity in the nuclei (Table 1). It would appear that there is a considerable pool of uridine precursors. Lack of information about the pool sizes in the different types of cell in the brain prevents a consideration of the effect of this factor on relative extents of incorporation into discrete classes of nuclei. The specific radioactivities of the precursors were kept constant in all experiments so that interpretation of results is not complicated by variation in this parameter.

The time-course of incorporation of 5 μ Ci of [³H]-uridine (sp. radioactivity 27 Ci/mmol) is shown in Table 1. Incorporation reached a maximum between 45 min and 1 h and then began to decrease.

[³H]Uridine was always present in double-label experiments, and the incorporation of this isotope served as a standard to which the incorporation of other precursors could be referred.

The time-course of incorporation of [¹⁴C]orotic acid and [³H]uridine is shown in Table 1. The incorporation of [¹⁴C]orotic acid increased linearly for 1 h and was level for the next 2 h. There was then a gradual decrease until at 16 h after injection, radio-

Table 1. Incorporation of various radioactive precursors of RNA into unfractionated brain cell nuclei

For details see the text.

Radioactive precursor	Dose (μ Ci)	Sp. radioactivity	Time of killing of animals after injection	$10^6 \times$ Radioactivity (d.p.m./nucleus)
$[^3\text{H}]$ Uridine	5	27.0 Ci/mmol	45 min	1 620
	10	27.0 Ci/mmol	45 min	4 040
	20	27.0 Ci/mmol	45 min	10 870
	5	0.27 Ci/mmol	45 min	1 370
	5	2.7 Ci/mmol	45 min	1 590
	5	5.0 Ci/mmol	45 min	1 550
	5	27.0 Ci/mmol	45 min	1 530
	10	27.0 Ci/mmol	15 min	3 030
	10	27.0 Ci/mmol	30 min	3 280
	10	27.0 Ci/mmol	45 min	4 360
	10	27.0 Ci/mmol	1 h	4 690
	10	27.0 Ci/mmol	2 h	3 310
	10	27.0 Ci/mmol	3 h	2 920
	10	27.0 Ci/mmol	4 h	2 280
	10	27.0 Ci/mmol	16 h	1 810
$[^{14}\text{C}]$ Orotic acid	3	60.8 mCi/mmol	45 min	330
	5	60.8 mCi/mmol	45 min	480
	6	60.8 mCi/mmol	45 min	590
	3	60.8 mCi/mmol	15 min	120
	3	60.8 mCi/mmol	30 min	160
	3	60.8 mCi/mmol	45 min	275
	3	60.8 mCi/mmol	1 h	340
	3	60.8 mCi/mmol	2 h	360
	3	60.8 mCi/mmol	3 h	380
	3	60.8 mCi/mmol	4 h	210
	3	60.8 mCi/mmol	16 h	104
$[^{14}\text{C}]$ Adenosine	3	52.0 mCi/mmol	15 min	53
	3	52.0 mCi/mmol	30 min	67
	3	52.0 mCi/mmol	45 min	110
	3	52.0 mCi/mmol	1 h	137
	3	52.0 mCi/mmol	1½ h	119
	3	52.0 mCi/mmol	3 h	85
	3	52.0 mCi/mmol	16 h	65

activity was approx. 25% of the maximum value. With $[^3\text{H}]$ uridine incorporation was linear up to a maximum 1 h after injection. The decrease in incorporation was fairly rapid in the next 1 h and was then gradual, so that after 16 h the radioactivity was 38% of the maximum value. The time-course of incorporation of $[^{14}\text{C}]$ adenosine is shown in Table 1. As with $[^3\text{H}]$ uridine, maximum radioactivity was reached after 1 h. The decrease in radioactivity over the next 2 h was fairly rapid and was followed by a very gradual decrease until at 16 h after injection time, the radioactivity was 47.5% of that after 1 h. The profile was generally similar to that for $[^3\text{H}]$ uridine.

Rappoport *et al.* (1971) injected $[^{14}\text{C}]$ orotic acid into the cisterna magna of rats at 16 and 90 days of age and found that the RNA synthesized in the

16-day-old rat brain reached a plateau in 3 h and remained at this value for the next 17 h after injection. The 90-day-old rats rapidly incorporated orotic acid within the first 3 h after injection, followed by a progressive decrease in incorporation during the next 17 h after injection. Saborio & Alemán (1970) injected $[^{14}\text{C}]$ uridine subcutaneously and intraventricularly into 18-day-old rats and found a linear increase of incorporation with time up to 40 min with a plateau for the next 5 h. Adams (1966) injected $[^{14}\text{C}]$ orotic acid subarachnoidally and found that incorporation of the precursor into nuclear RNA in the cerebral cortex proceeded at similar rates in 4-day-old and adult rats. In both young and adult rats, incorporation proceeded linearly with time to a maximum at about 2 h in adults or 5 h in 4-day-old rats, and

Table 2. *Incorporation in vivo of [³H]uridine and in vitro of [³H]UTP into RNA of rat brain nuclei fractionated by zonal centrifugation, and ratios of the radioactivities in each peak to those in zone (II)*

In experiments *in vivo*, 11 rats (90 g, male albino) were each injected with 10 μ Ci of [³H]uridine (sp. radioactivity 27 Ci/mmol). After 30 min animals were killed and the brain nuclei isolated and fractionated (see the Experimental section). In experiments *in vitro*, 14 animals were used and the radioactivity of RNA polymerase with [³H]UTP as precursor and 3 mM-MnCl₂ and 0.3 M-(NH₄)₂SO₄ in the incubation medium was measured as described previously (Austoker *et al.*, 1972). Radioactivity in both cases is expressed as 10⁶ $\times$ d.p.m./nucleus.

Zone	[³ H]Uridine		[³ H]UTP	
	Radioactivity	Ratio	Radioactivity	Ratio
(I)	5413	0.91	250	0.94
(II)	5940	1.00	267	1.00
(III)	4661	0.79	157	0.59
(IV)	2966	0.50	119	0.45
(V)	1423	0.23	113	0.42

decreased to about 80% of this value after 22 h. These results are generally in agreement with our findings with young adult rats (approx. 45 days old).

It would be interesting to study the extent of incorporation of the various precursors into the cytoplasmic RNA as a function of time to determine whether the fall off in the extent of incorporation is accompanied by a corresponding increase in the radioactivity of the cytoplasmic RNA. Balázs & Cocks (1967) calculated the percentage distribution of [¹⁴C]orotic acid in the RNA of their crude nuclear pellet (post-800g pellet), various subcellular fractions, and also in the cytoplasmic RNA (represented by the nuclei-free supernatant). After 30 min, 87% of the ¹⁴C was in the crude nuclei, after 1½ h 84%, after 3 h 67%, and after 16 h 58% was in cytoplasmic RNA and 42% in the crude nuclei. They concluded that incorporation into the cytoplasmic RNA was very slow during the first 1½ h after injection. This suggests, in agreement with Prescott (1964), that the nucleus is the main site of RNA synthesis in the brain.

Studies with fractionated nuclei

The relative incorporation of the different classes of nuclei separated by zonal centrifugation, with [³H]uridine (*in vivo*) and with [³H]UTP (*in vitro*) as precursors are shown in Table 2. In both cases the incorporation in zone (II) (astrocytes I) was greatest. Taking this value as 1 the ratio of the incorporation in each peak relative to that in zone (II) was calculated (Table 2). The most striking difference between the findings *in vivo* and *in vitro*, was that the radioactivity of oligodendrocytes (I) (zone IV) was more than twice that of oligodendrocytes (II) (zone V) *in vivo*, whereas *in vitro* virtually no difference could be detected between these two classes. Previously, the only differences noted between these classes of oligodendrocytes

was the smaller volume of the nuclei in zone (V). The lower radioactivity in the oligodendrocytes (II) in zone (V) was in keeping with their smaller size and correspondingly more compacted chromatin, although such a large difference in radioactivity is not predictable. Another difference is that the astrocytes (II) (zone III) showed a higher radioactivity *in vivo*, closer to that of astrocytes (I) (zone II), in comparison with their relative radioactivities *in vitro*.

In the first set of experiments the period of incorporation was 30 min. The possibility existed that the kinetics of incorporation of radioactive precursors by the different classes of nuclei would not be uniform. In addition it was necessary to examine the uptake of radioactive precursors of RNA other than [³H]uridine. To eliminate any variability due to differences between individual animals a comparison of alternative precursors with uridine was always carried out by using the double-label technique with incorporation of [³H]uridine as the standard. The pattern of incorporation of [³H]uridine into different classes was consistent. A time-course of incorporation over a period of up to 16 h was examined, by using [¹⁴C]orotic acid and [³H]uridine as precursors (Fig. 1). With both precursors, and in all five classes of nuclei, radioactivity was approximately linear with time for the first 45 min.

With [³H]uridine (sp. radioactivity 27 Ci/mmol) as precursor, maximum activity in zones (I) and (IV) was reached after 1½ h and radioactivity then gradually declined to 40–60% of maximum radioactivity after 16 h. In zone (V), the maximum radioactivity was reached between 45 min and 1 h. At all times the radioactivity in zone (IV) was considerably greater than that in zone (V). However, the radioactivity of astrocytes (II) (zone III) which was 72% that of astrocytes (I) (zone II) after 1½ h, was more or less the same after 16 h. The order of radioactivities of the

Fig. 1. Kinetics of RNA synthesis *in vivo* in fractionated brain nuclei with $[^3\text{H}]$ uridine (a) and $[^{14}\text{C}]$ orotic acid (b) as precursors

For each time of incorporation, 11 rats were each injected with $3\mu\text{Ci}$ of $[^{14}\text{C}]$ orotic acid (sp. radioactivity 60.8mCi/mmol) and $10\mu\text{Ci}$ $[^3\text{H}]$ uridine (sp. radioactivity 27Ci/mmol). The brain nuclei were prepared and fractionated as described in the Experimental section. $\square$, Zone (I); $\circ$, zone (II); $\bullet$, zone (III); Δ , zone (IV); $\blacktriangle$, zone (V).

classes of nuclei was the same with $[^3\text{H}]$ uridine as that found *in vitro* with $[^3\text{H}]$ UTP as precursor, with zone (II) showing the highest radioactivity. During the period of maximum incorporation *in vivo* (30 min–3 h)

astrocytes (II) showed consistently higher radioactivity *in vivo* than they did *in vitro* relative to astrocytes (I). Similarly oligodendrocytes (II) showed a consistently lower specific radioactivity than that of oligodendrocytes (I), so that even at the time of maximum radioactivity for this class of nuclei *in vivo* (approx. $\frac{1}{2}$ h) its radioactivity was approximately half that of oligodendrocytes (I).

The higher radioactivity in astrocytes and neuronal nuclei with $[^3\text{H}]$ uridine as precursor is similar to the findings of Lovtrup-Rein (1970) that the rate of incorporation of $[^3\text{H}]$ adenine and $[^3\text{H}]$ cytidine into nuclear RNA of rat brain was four times higher in astrocytes and neuronal nuclei than the other glial nuclei.

With $[^{14}\text{C}]$ orotate as precursor (Fig. 1), the overall profiles of incorporation are similar to those with $[^3\text{H}]$ uridine, in that maximum radioactivity in zones (I) and (IV) was reached after $1\frac{1}{2}$ h, whereas in zone (V) it was reached after 45 min to 1 h. However, there are considerable differences in the relative radioactivities of the classes of nuclei. Zone (I) showed the greatest radioactivity over the first 3 h and the nuclei in zone (III) had a higher radioactivity for the first 2 h than those in zone (II). The radioactivity in zone (IV) was considerably higher relative to zones (I)–(III) compared with the findings *in vivo* with $[^3\text{H}]$ uridine as the precursor, or the findings *in vitro*. The radioactivity in zone (IV) was nearly four times that in zone (V) (after $1\frac{1}{2}$ h). Zone (III) was clearly more active over the first 3 h in incorporation of $[^{14}\text{C}]$ orotate than is zone (II), and zone (IV) had a much higher rate of incorporation of $[^{14}\text{C}]$ orotate as compared with $[^3\text{H}]$ uridine when comparing this zone to zones (I)–(III). Again the considerable differences between the two classes of oligodendrocytes are apparent. It is noteworthy that Hogans *et al.* (1971) demonstrated that uridine was superior to orotic acid in labelling RNA in incubated slices of rat brain, whereas it was equally effective in hepatic and renal slices.

The incorporation of $[^{14}\text{C}]$ adenosine into fractionated nuclei from brain cells at 45 min and 4 h after injection of the tracer is shown in Table 3. At both times the nuclei in zone (I) showed a higher value of radioactivity than those in zone (II). This was in contrast with the findings with $[^3\text{H}]$ uridine as a precursor, where zone (II) showed greater radioactivity. It was also in contrast with the higher rate of RNA synthesis shown by the nuclei in zone (II) *in vitro*, compared with that in zone (I) with $[^3\text{H}]$ ATP as precursor (Austoker *et al.*, 1972). David *et al.* (1971) have demonstrated an enzyme that polymerizes ATP in rat brain nuclei. It is possible that the neuronal nuclei (zone I) have a higher rate of poly(A) formation *in vivo*, than the other classes of nuclei. Daneholt & Brattgård (1966), however, have shown a higher rate of incorporation of $[^3\text{H}]$ -adenine into glial cells *in vivo*, and of $[^3\text{H}]$ cytidine

Table 3. *RNA synthesis in fractionated rat brain nuclei in vivo with [¹⁴C]adenosine as precursor*

At each time 11 rats were injected with 3 μ Ci of [8-¹⁴C]adenosine (sp. radioactivity 52mCi/mmol) and 10 μ Ci [5-³H]uridine (sp. radioactivity 27Ci/mmol); the latter was a standard of radioactivity. After 45 min or 4 h the animals were killed and the nuclei prepared and fractionated (see the Experimental section).

Zone	Time of killing of animals after injection	...	10 ⁶ $\times$ Radioactivity (d.p.m./nucleus)	
			45 min	4 h
(I)			393	269
(II)			282	184
(III)			211	122
(IV)			125	120
(V)			100	80

into the nerve cells of rabbit. It is noteworthy that in the formation of poly(A) *in vitro* in fractionated nuclei from brain cells, neuronal nuclei (zone I) showed a higher activity than those of astrocyte nuclei (zone II) (Austoker *et al.*, 1972).

Again a difference in radioactivity between the two classes of oligodendrocyte nuclei was apparent, although it was not as pronounced as that with either [³H]uridine or [¹⁴C]orotic acid as precursors. The radioactivity after 4 h had declined more rapidly in zones (I) and (II) than in zones (IV) and (V). This was similar to the finding with [³H]uridine as precursor.

Conclusions

In general there is a parallel between the synthesis of RNA *in vivo* and *in vitro* although some differences do exist. The most striking of these is that a significant difference between the radioactivities of the two classes of oligodendrocyte nuclei is apparent *in vivo*, but not *in vitro*. This difference was found with all three radioactive precursors used *in vivo*, but not with any of the precursors used *in vitro*. In addition, astrocytes (II) (zone III) showed higher radioactivity *in vivo*, closer to that of astrocytes (I) (zone II), than *in vitro*. The overall radioactivities of the classes of nuclei can be related to their morphological differences. The nuclei of zones (I) and (II) (neuronal and astrocyte I nuclei) with a predominance of disperse, lightly staining chromatin (euchromatin) showed a higher incorporation of radioactivity than astrocytes (II) (zone III) or oligodendrocytes (I) and (II), which with dense tightly packed chromatin (heterochromatin) would be expected to show less incorporation of radioactivity. The oligodendrocytes (II) (zone V) are smaller than oligodendrocytes (I) (zone IV) and their lower incorporation of radioactivity *in vivo* is probably a consequence of the even more compact nature of their chromatin. The much higher radio-

activity found in neuronal and astrocyte nuclei than in oligodendrocyte nuclei was in agreement with the work of Løvtrup-Rein (1970). The relative inertness of small condensed nuclei is also observed both *in vivo* and *in vitro* in the comparison of the stromal and parenchymal nuclei of rat liver (Johnston & Mathias, 1972).

The time-course of incorporation with different precursors showed a similar profile in all classes of nuclei although some differences do exist. In general, radioactivity reached a maximum between 30 min and 2 h and then showed a steady decrease. This was in agreement with the work of other authors.

The amount of radioactivity in the nuclei at any time will be a balance of several processes. The rate of synthesis of RNA is related to the pool size and specific radioactivity of the radioactive precursors as well as to the activity of RNA polymerase. There is an uncertainty attached to the relative incorporations because of a lack of knowledge of pool sizes in each type of cell in the brain. Such information is not accessible. This prevents a direct comparison between incorporations of purines and pyrimidines *in vivo* into the different classes of nuclei, so that one can consider the incorporations only on a relative scale. It is noteworthy that whereas zone (I) contains more radioactivity than zone (II) after injection of [¹⁴C]-adenosine, the reverse is true with [³H]uridine. Whether this is due to a high rate of poly(A) synthesis in neuronal nuclei or not, is uncertain, although the higher rate of poly(A) synthesis *in vitro* in neuronal nuclei (Austoker *et al.*, 1972) makes this seem likely. Other factors affecting the amount of radioactivity in the nuclei will be the rate of degradation within the nucleus, and the rate of export of RNA from the nucleus to the cytoplasm. After a single injection, the amount of radioactivity within the nucleus remains at about 40–60% of maximum radioactivity up to about 16 h after injection. Reutilization of the label may account for a considerable part of this.

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