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The Relationship Between Cellular Nucleic Acids in the Developing Rat Cerebral Cortex

By D. H. ADAMS

Department of Biochemistry, Institute of Psychiatry (British Postgraduate Medical Federation, University of London), Maudsley Hospital, Denmark Hill, London, S.E. 5

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1. In the rat cerebral cortex net DNA synthesis ceases when the animal has reached about 25g. body weight (18 days of age). There is then little further change in the DNA content per cortex. 2. Nuclear and transfer RNA follow a similar pattern to DNA. 3. Microsomal and ribosomal RNA content increases up to 25g. body weight but then declines. The decrease in ribosomal and microsomal RNA content is associated with a change in RNA base composition. 4. Incorporation of [^{14}C]orotic acid into nuclear RNA proceeds at a similar rate in 4-day-old and adult animals. However, there is a lag period of about 60min. in the young animals during which incorporation into the ribosome fractions proceeds slowly. In the adult animals the lag period is not seen.

There have been many observations supporting the hypothesis that ribosomal RNA is first formed in the nucleus. According to McCarthy, Britten & Roberts (1962) in bacterial systems the newly formed RNA enters the cytoplasm in the form of 8–20s sub-units (eosomes), complete ribosomes subsequently being formed by aggregation. In a review of the literature Prescott (1964) concluded that ribosomal RNA arises solely from nuclear precursors. The principal opposition to this view has come from H. Harris and his group (Harris, 1959; Watts & Harris, 1959; Harris & Watts, 1962; Harris, Fisher, Rodgers, Spencer & Watts, 1963; Watts, 1964), who, working mainly with HeLa cells, have concluded that the rapidly synthesized nuclear RNA is rapidly broken down *in vitro* and find little or no evidence that it is transferred directly into the cytoplasm. Singh & Koppelman (1963) claimed that the results of Harris and co-workers were in fact compatible with a precursor relationship between nuclear and cytoplasmic RNA, but this was disputed by Harris (1963).

Much of the evidence for a nuclear RNA–cytoplasmic RNA precursor relationship is based on observations that labelled precursors enter nuclear RNA very rapidly whereas there is a lag period before the label appears in ribosomal RNA. This lag period appears to be extremely variable and may be only a matter of a few minutes in *Neurospora* (Zalokar, 1960) to several hours in cultured *Triturus* cells (Prescott, 1964). Prescott (1964) has suggested that the length of the lag period increases from cell to cell roughly in proportion to the length of the cell life cycle. Watts (1964) has also shown that the lag period can be altered by external factors. It could virtually be abolished in HeLa cells by allowing them to grow in a purine-free medium for 24hr. before precursor administration.

So far, however, very little work appears to have been done on the relationship between cellular nucleic acids in adult mammalian tissue. Adams (1965) in a study of [^{14}C]orotic acid incorporation into weanling and young-adult rat-brain RNA found that, when injected intracisternally, this compound became rapidly incorporated into all RNA fractions. The data suggested that there was little if any lag period between nuclear and ribosomal labelling.

The present paper describes further experiments related to this observation.

MATERIALS AND METHODS

Animals. Wistar albino rats bred in this Institute were used when 3–4 days of age (weight about 7g.) or as adults weighing 200–250g. In one experiment rats of intermediate sizes were also used.

Radioactive isotope. [^{14}C]Orotic acid was obtained from The Radiochemical Centre, Amersham, Bucks. It was diluted before use with unlabelled material to a specific activity 10μc/μmole, dissolved in 0·9% NaCl and injected into the subarachnoidal space (Lindberg & Ernster, 1949). Normally 4-day-old rats received 2μc (0·02ml.) and adults 4μc (0·04ml.).

Preparation of homogenates and subcellular fractions. Rats were killed by decapitation, the brains rapidly removed and the cerebral cortices dissected out. Material from three to five rats was pooled for each determination. Homogenization was effected by placing 1 part of the brain

together with 10–20 parts of 0·32M-sucrose containing 0·5mM-$MgCl_2$ (pH 7·2) in a glass homogenizer with a Teflon pestle, and rapidly moving the pestle up and down nine times. The fractions were collected by centrifuging as follows:

(1) 1000*g* for 5min. in a Mistral 6L refrigerated centrifuge (nuclear fraction). The pellet was gently resuspended in 10ml. of fresh sucrose and recentrifuged, the supernatant being discarded.

(2) The original supernatant from (1) was centrifuged at 10000*g* for 10min. in the no. 30 head of a Spinco model L ultracentrifuge, and the pellet discarded.

(3) The supernatant from (2) was centrifuged in the Spinco ultracentrifuge at 20000*g* for 1hr. The pellet (microsome fraction) was resuspended in fresh sucrose and recentrifuged.

(4) The supernatant from (3) was again centrifuged in the Spinco ultracentrifuge (40 head) at 105000*g* for 2hr. (ribosome fraction), the soluble fraction remaining in the supernatant.

The pellets were resuspended in 0·9% NaCl and stored at −20° until they were processed.

Extraction of DNA and RNA. This was done by the hot 10% NaCl method of Tyner, Heidelberger & LePage (1953), the lipid-extraction step being modified according to Adams (1965). The extracted nucleic acids were washed three times at −5° with ethanol (90%, v/v). All fractions were then submitted to mild alkaline hydrolysis in 0·1N-NaOH at 37° for 24hr. and then made 0·1N with respect to HCl. After standing at room temp. for 1hr. the preparations were centrifuged at 3000*g* for 10min. and any precipitate was dissolved in 0·1N-NaOH and estimated for DNA. Normally DNA was found only in the nuclear fraction.

Estimation of nucleic acids. RNA was estimated by the orcinol method (Hurlbert, Schmidt, Brumm & Potter, 1954) and DNA by the diphenylamine reaction (Dische, 1955).

RNA base composition. This was determined by heating the alkaline hydrolysates of RNA in N-HCl for 1hr. at 100°. Samples were run on paper (Whatman no. 1) for 48hr. with 70% (v/v) 2-methylpropan-2-ol made 0·8N with respect to HCl as solvent. The adenine, guanine, uridylic acid and cytidylic acid spots were detected under ultraviolet light, cut from the paper and eluted with N-HCl at 38°.

The resulting solutions were read at their respective absorption maxima in a Unicam SP.500 spectrophotometer, and are uncorrected.

RESULTS

Age variation of the nucleic acid content of the cerebral cortex. The DNA content, and the RNA content of the various cell fractions, was estimated at intervals from 3 to 4 days of age (7g. body wt.). The average content per cortex of the various nucleic acids and the cortex weight, plotted against total body weight, are given in Fig. 1. This shows that the total DNA and nuclear and transfer RNA present per cortex increased up to about 25g. body wt. and then remained relatively constant. The ribosomal and microsomal RNA content also increased up to 25g. but then declined.

Orotic acid-incorporation studies. Fig. 2(*a*) shows the specific activity of RNA and DNA isolated from 4-day-old rat cerebral cortex after the injection of 2μc of [^{14}C]orotic acid, and Fig. 2(*b*) shows the results of a similar experiment in which adult rats (wt. 200–250g.) were injected with 4μc of [^{14}C]orotic acid. The labelling of the ribosomal

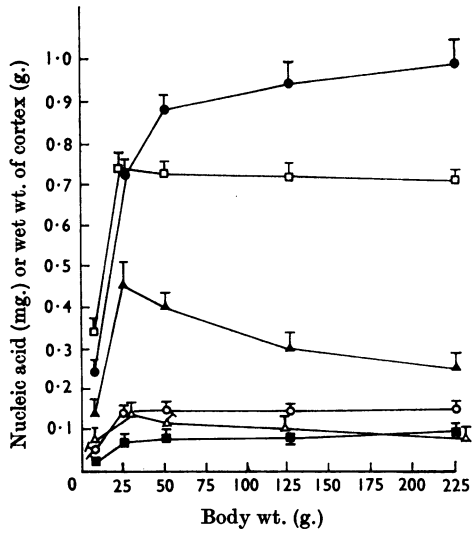

Fig. 1. Wet weight, DNA content and RNA content of subcellular fractions of the rat cerebral cortex during development. The nucleic acid values are the average amounts present in one whole cortex taken from rats of the stated body weights, as arithmetic means + S.E.M. (bars). Each point corresponds to six to eight estimations. ●, Wet wt. (g.); □, DNA content (mg.); ▲, ribosomal RNA content (mg.); △, microsomal RNA content (mg.); ○, nuclear RNA content (mg.); ■, transfer RNA content (mg.).

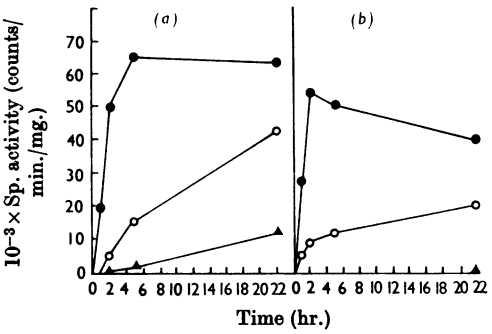

Fig. 2. Incorporation of [^{14}C]orotic acid label injected subarachnoidally into nucleic acids of the rat cerebral cortex. (*a*) 4-day-old rats (given 2μc); (*b*) adult rats (given 4μc). ●, Nuclear RNA; ○, ribosomal RNA; ▲, DNA.

RNA in both groups over the first 2hr. is shown on an expanded scale in Fig. 3.

In the adult animals a very slow incorporation of label into DNA was observed, but this reached only 200 counts/min./mg. after 24hr. compared with 12000 counts/min./mg. in the young.

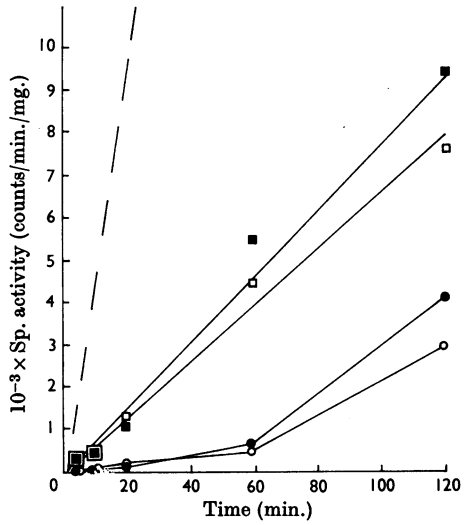

Fig. 3. Incorporation of [^{14}C]orotic acid into the ribosomal and microsomal RNA of rat cerebral cortex over the 5–120min. period. Details are as given in Fig. 2. Incorporation into nuclear RNA is represented by the broken line. Adult rats: ■, ribosomal RNA; □, microsomal RNA. 4-day-old rats: ●, ribosomal RNA; ○, microsomal RNA.

The ratios of incorporation into nuclear RNA/incorporation into ribosomal RNA for the two groups over the whole period are given in Table 1, and show that over the first 60min. this ratio was of the order of 40 for the young animals and 6 for adults. It seemed possible that this difference might be due to differences in rates of diffusion of label into the brain mass. To check this possibility adult rats were injected intracisternally with 4μc of [^{14}C]orotic acid, and the nuclear RNA/ribosomal RNA incorporation ratio was determined in cerebral cortex, cerebellum and white matter. Adams (1965) has shown that, when this route of injection was used, much more of the injected material becomes available to the cerebellum and white matter, than to the cerebral cortex, and the rates of diffusion into the three types of tissue should therefore be very different.

The incorporation into nuclear RNA and ribosomal RNA and the nuclear/ribosomal ratio over the 5–20min. period are shown in Table 2. Although there was a considerable difference in the specific activities of the three RNA samples the incorporation ratio was reasonably constant and similar to the adult figures in Table 1.

RNA base composition. In view of the observation (Fig. 1) that the ribosomal and microsomal RNA content of the cerebral cortex decreased by about

Table 1. *Ratios of incorporation of* ^{14}C *into rat-brain nuclear-fraction RNA to incorporation into microsoma-fraction RNA (N/M) or ribosomal-fraction RNA (N/R) after subarachnoid injection of* [^{14}C]*orotic acid*

	Time after injection						
	5 min.	10 min.	20 min.	60 min.	2 hr.	5 hr.	24 hr.
N/M							
Adult	5·6	6·4	8·0	7·5	7·3	4·0	1·7
4-day-old	18	34	41	36	12	4·6	1·8
N/R							
Adult	5·4	5·8	10·0	5·8	5·8	3·3	1·6
4-day-old	40	47	53	32	16	5·2	2·1

Table 2. *Incorporation of intracisternally injected* [^{14}C]*orotic acid into nuclear (N) and ribosomal (R) RNA of adult rat cerebral cortex, cerebellum and white matter*

Results are given in counts/min./mg. of RNA. 4μc (10μc/μmole) of orotic acid was injected into each rat.

	Time after injection								
	5 min.			10 min.			20 min.		
	N	R	N/R	N	R	N/R	N	R	N/R
Cerebral cortex	140	25	5·6	260	50	5·2	620	85	7·3
Cerebellum	350	80	4·4	1100	150	7·3	2700	325	8·3
White matter	450	70	6·4	780	100	7·8	1950	250	9·8

Table 3. *Base composition of RNA samples isolated from rat cerebral cortex*

Results are given as moles/100 moles ± S.E.M. and are uncorrected for guanine hydrolysis. The numbers of observations per figure are given in parentheses. The differences between the guanine and cytosine contents of the ribosomes from adult and 4-day-old animals are highly significant ($t > 4$ for 21 degrees of freedom).

	Guanine	Adenine	Cytosine	Uracil
Nuclear				
Adult (8)	30·5 ± 0·67	24·1 ± 0·42	26·4 ± 0·59	19·0 ± 0·48
4-day-old (10)	30·7 ± 0·42	24·2 ± 0·59	25·2 ± 0·77	19·9 ± 0·50
Microsomal				
Adult (10)	31·7 ± 0·49	19·5 ± 0·35	29·6 ± 0·47	19·2 ± 0·67
4-day-old (10)	33·6 ± 0·47	20·5 ± 0·18	27·9 ± 0·50	18·0 ± 0·31
Ribosomal				
Adult (13)	31·2 ± 0·35	19·7 ± 0·34	30·0 ± 0·37	19·1 ± 0·68
4-day-old (10)	33·7 ± 0·59	21·0 ± 0·38	27·7 ± 0·54	17·6 ± 0·62

40% as the animals increased in body weight from 25 to 225 g. estimations of base compositions were made in 4-day-old and adult animals. The base composition of nuclear RNA was also determined in the two groups, and the results are given in Table 3.

DISCUSSION

The results of Fig. 1 have shown that in the rat cerebral cortex net DNA synthesis ceased comparatively early (about 18 days of age, 25 g. body wt.). The net synthesis of all cellular RNA ceased at about the same point, and this was followed in the microsomal and ribosomal RNA by a decrease of nearly 40% by the time the animals reached 200–250 g. body wt. It seems reasonable to suppose that this RNA decrease represents a decline in the microsome–ribosome population.

The subsequent studies were therefore made on the cerebral cortex of 3–4-day-old rats in which net synthesis of all nucleic acids was proceeding, and of adult (200–250 g.) rats in which there was no net synthesis and a declining microsome–ribosome population. Preliminary experiments with [^{14}C]-orotic acid showed that if the adult animals were injected with 4 μc and the 4-day-old animals with 2 μc the incorporation of label into the nuclear RNA of both groups was approximately linear and at the same rate for 2 hr. Since the cortex of the adult was three to four times as large as that of the 4-day-old, this suggested that there was no great age difference in ability to incorporate label into nuclear RNA.

The rapidity and similar rate of the nuclear incorporation in both groups suggests that most of the nuclear RNA is undergoing a rapid intracellular turnover, particularly in the adult animals in which there was no net synthesis of RNA. This is in substantial agreement with the work of Watts (1964).

In each group the pattern of incorporation of label into microsomal and ribosomal RNA was very similar, but very different in the two groups. In the 4-day-old animals there was very little ribosomal labelling in the first 60 min. and then a rapid increase, whereas the ribosomal labelling in the adults was essentially linear from 5 to 120 min. and when extrapolated cut the time axis at +3 min., which is closely similar to the intersection of the plot of the nuclear incorporation. These results suggest that the lag period between nuclear and ribosomal incorporation is abolished in the adult cells. The interpretation of the results is complicated by lack of knowledge of the nature of the labelled RNA associated with the ribosomes, in particular whether it is all of a 'messenger' type in the two groups. However, the results obtained with young brain seem consistent with the usual view of a precursor relationship between nuclear and cytoplasmic RNA. A similar conclusion has recently been reached by Singh (1965) through a kinetic analysis of precursor incorporation into young brain. The situation in the adults certainly seems dissimilar, and the results suggest two possibilities. Either in the adults the ribosomal RNA has become self-replicating, or precursors are entering the cytoplasm from the nucleus at a greatly increased rate. However, in view of the declining ribosome population in the adult this second alternative seems less likely.

The results of Watts (1964) showing that the lag period between nuclear and cytoplasmic incorporation in HeLa cells could be abolished by pre-incubation of the cells in a purine-free medium were interpreted by this author in terms of a diminished intracellular purine pool size permitting more rapid precursor incorporation into cytoplasmic RNA. This possible reason for change in lag period seems unlikely to bear on the present results since the total uridine nucleotide pool is only about 20% lower in the adult than in the newborn rat cerebral cortex (Adams, 1965).

The base composition of nuclear RNA from both young and adult cortex was similar and the RNA

contained 56–57% of guanine–cytosine. Microsomal and ribosomal RNA from each group had a similar base composition, but although they had the same guanine–cytosine content of slightly over 61%, young microsomes and ribosomes contained significantly more guanine and less cytosine than adult. The difference in guanine–cytosine content between nuclear and ribosomal RNA could be explained if the nucleus contained about 20% of a messenger-type RNA with about 42% of guanine–cytosine. The difference in base ratio between microsomes and ribosomes from young and adult animals suggests that they consist of different populations, and therefore that a specific fraction is lost in the declining adult population.

It might be suggested that the lost fraction is that concerned with the additional protein requirements of the dividing cell, the fraction remaining in the adult cell being principally concerned with the synthesis of protein for cell maintenance.

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