Re-utilization of pyrimidine nucleotides during rat liver regeneration

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The changes in the specific radioactivities of the pool of total acid-soluble uridine nucleotides and of uridine and cytidine components of total cellular and nuclear RNA were monitored in regenerating rat liver for 12 days after partial hepatectomy. Evidence is presented for the re-utilization of pyrimidine nucleotides derived from cytoplasmic RNA degradation for the synthesis of new RNA. The extent of recycling was assessed and the true rate of rRNA turnover determined more accurately. The re-utilization of the uridine components of RNA was 7.0%/day during the proliferative and 3.2%/day during the post-proliferative phase, whereas that of the cytidine nucleotides was more pronounced (9.6%/day and 18.1%/day respectively). The results reveal the existence of partial compartmentalization of pyrimidine ribonucleoside triphosphate pools in the nucleus and cytoplasm of rat liver cells.

The basic strategy in measuring the rRNA turnover in rat liver is pulse-labelling it with a suitable precursor and then monitoring the decay of radioactivity in rRNA moieties. In all studies that employ labelled orotate it is assumed that there is no re-utilization of radioactive rRNA degradation products into newly synthesized rRNA, and that even if it occurs it has a minimal effect upon the observed turnover rates (Hirsch & Hiatt, 1966; Wilson & Hoagland, 1967; Tsurugi *et al.*, 1974; Loeb & Yeung, 1975; Eliceiri, 1976; Retz & Steele, 1980). Our own more-recent turnover data for regenerating and normal rat liver (Nikolov *et al.*, 1983; Nikolov & Dabeva, 1983) are based on this assumption too.

However, re-utilization of pyrimidine nucleotides originating from degradation of cellular RNA has been suggested to occur (Blobel & Potter, 1968; Genchev, 1980). Upon labelling of rat liver rRNA with [¹⁴C]orotate different turnover rates were observed for the uridine and cytidine components of rRNA (Seifert & Vacha, 1974). Melvin *et al.* (1976) obtained different half-lives for kidney rRNA on labelling with different precursors. Even re-utilization of preformed pyrimidines (mainly

cytidine nucleotides) for the synthesis of DNA was observed (Seifert, 1982).

The question of re-utilization of pyrimidine nucleotides for the synthesis of new RNA in eukaryotes is further complicated by the uncertainty about the compartmentalization of the ribonucleotide precursor pools, i.e. whether the specific radioactivity of the acid-soluble nucleotide pool extracted from the whole cell is identical or not with the specific radioactivity of the ribonucleotide triphosphate pool utilized for nRNA synthesis.

In some cases evidence was obtained that there is one ribonucleotide pool serving the synthesis of all RNA species (Wu & Soeiro, 1971; Soeiro & Ehrenfeld, 1973; Brandhorst & McConkey, 1974; Puckett & Darnell, 1977). However, in other studies there are various lines of evidence leading to the opposite conclusion: the existence of separate cytoplasmic (for the synthesis of hnRNA) and nuclear (for rRNA and tRNA formation) pyrimidine pools (Plagemann, 1971, 1972; Wieggers *et al.*, 1976; Genchev *et al.*, 1980; Falkenthal & Lengyel, 1981; Birch & Turnock, 1982). This controversy was partially resolved by Khym *et al.* (1978) showing a rapid equilibration between the compartmentalized pools.

In the present investigation we studied the changes in the specific radioactivity of the whole-cell pool of uridine nucleotides in regenerating rat liver and showed that re-utilization of RNA degradation products (UMP and mainly CMP)

Abbreviations used: pre-rRNA, precursor of rRNA; hnRNA, heterogeneous nuclear RNA; RNA-UMP and RNA-CMP, uridine and cytidine components respectively of total cellular RNA; nRNA-UMP and nRNA-CMP, uridine and cytidine components respectively of nRNA.

occurs in both regenerating and normal rat liver. A correction for recycling of radioactive precursors arising from RNA breakdown was made in order to determine the true rate of rRNA turnover more accurately during the proliferative and post-proliferative phases of liver regeneration.

Experimental

Animals and cell fractionation

The experiments were carried out with Wistar male albino rats. Labelling *in vivo*, partial hepatectomy and homogenation of liver were performed as described previously (Nikolov *et al.*, 1983; Nikolov & Dabeva, 1983). Briefly, [2-¹⁴C]orotate was administered intraperitoneally in four 50 μ Ci portions per rat every 12 h, and 5 days after the last injection the animals were partially hepatectomized. The relative increase of the amount of rRNA in regenerating liver was determined (eqn. 2 below) in a parallel experiment in which weanling rats of 55–65 g body wt. were injected with ten 15 μ Ci portions of [6-³H]thymidine per rat every other day and partial hepatectomy was performed 9 days after the last injection. All the animals were killed in groups of three at intervals of 1 to 12 days after the operation. The weight range of the animals at the time of operation was 180–215 g. Livers for each time point were processed individually. The excised lobes were used as a zero-time point for the respective regenerating liver.

Purified nuclei were isolated by the hyperosmotic-sucrose/Triton X-100 method (Dabeva *et al.*, 1978) from liver homogenate, and nRNA was extracted by the phenol method.

Analysis of pyrimidine nucleotides of the acid-soluble pool

For determination of specific radioactivity of the pyrimidine components of the whole-cell nucleotide pool about 1.5 g of liver was homogenized in 9 vol. of ice-cold 0.6 M-HClO₄. After standing for 1 h on ice and centrifugation at 6000 g for 15 min, the sediment was extracted once more with the same volume of 0.2 M-HClO₄. The pooled acid-soluble extracts were adjusted to 1 M-HClO₄ and heated for 30 min at 95°C before adsorption on charcoal and subsequent elution with 2% (v/v) conc. NH₃ in 50% (v/v) ethanol. The samples were dried *in vacuo* and dissolved in 0.05 M-HCl, and UMP and CMP were separated by cation-exchange chromatography on columns (8 cm $\times$ 0.9 cm) of Dowex 50 X4 (H⁺ form; 200–400 mesh) (Katz & Comb, 1963). UMP was quantitatively washed through the column with 6 ml of 0.05 M-HCl. Afterwards water was used as eluent, six 5 ml fractions being collected. The bulk of CMP was reproducibly eluted in the last three

fractions, which were acidified with 50 μ l of 4 M-HCl. UMP and CMP were concentrated by charcoal treatment and elution as described above. Finally, after being dried *in vacuo*, they were dissolved in water, spotted on PEI- (polyethyleneimine-) cellulose plates and developed with 1 M-acetic acid up to 7 cm from the origin, followed by 0.3 M-LiCl (Randerath & Randerath, 1967). The UMP and CMP spots were identified by using 5'-nucleotide markers and confirmed by autoradiography. This procedure was found to clearly separate 5'-UMP from 2'(3')-UMP. The nucleotides were eluted with 0.7 M-MgCl₂/0.02 M-Tris/HCl buffer, pH 7.4, and their specific radioactivities were determined as d.p.m./ μ mol of nucleotide. Molar absorption coefficients of $\epsilon_{262} = 9.90 \times 10^3 \text{ M}^{-1} \cdot \text{cm}^{-1}$ for UMP and $\epsilon_{260} = 7.60 \times 10^3 \text{ M}^{-1} \cdot \text{cm}^{-1}$ for CMP were used.

Nucleotide analysis of RNA

The pyrimidine components of total cellular RNA and of nRNA were analysed on PEI-cellulose (see above) after hydrolysis with 0.3 M-KOH for 18 h at 37°C and subsequent acidification and adsorption on charcoal.

Calculations

The actual total radioactivity in RNA at time t [$R^*(t)$] can be determined from the equation:

$$R^*(t) = R(t) - R^p(t) \quad (1)$$

where $R(t)$ is the measured radioactivity and $R^p(t)$ is the radioactivity contributed by the recycling of labelled nucleotides into newly synthesized RNA. They are given by:

$$\begin{aligned} R(t) &= S(t) \cdot A(t) \\ R^p(t) &= [A(t) - A(0)] S^p \end{aligned}$$

where S^p denotes the average specific radioactivity of the UTP pool from which the increment of RNA [$A(t) - A(0)$] is synthesized. The behaviour of the specific radioactivity of the precursor pool is described by:

$$S^p(t) = S^p(0) \cdot e^{-k^p t}$$

and the fit of the data to an exponential decay is shown to be reasonably good (see Fig. 1); $S(t)$ and $S(0)$ (d.p.m./ μ g of RNA) are the specific radioactivities of RNA at time t and at the time of partial hepatectomy respectively; $A(t)$ is the amount of rRNA in the regenerating liver and $A(0)$ is the rRNA amount in the initial liver remnant. The relative increase of rRNA amount in the regenerating liver [$A(t)/A(0)$] after a time interval t is calculated from:

$$\frac{A(t)}{A(0)} = \frac{r(t) \cdot s^+(0)}{r(0) \cdot s^+(t)} \quad (2)$$

where $r(0)$ and $r(t)$ are the rRNA/DNA ratios and $s^{\dagger}(0)$ and $s^{\dagger}(t)$ (d.p.m./ μg of DNA) are the specific radioactivities of DNA in the initial (excised) liver and regenerating liver at time t respectively. For further details see Nikolov *et al.* (1983) and Nikolov & Dabeva (1983). Substituting these values in eqn. (1) and dividing by $A(t)$, then the corrected specific radioactivity of RNA [$S^*(t)$] at time t after partial hepatectomy will be:

$$S^*(t) = S(t) - [1 - A(0)/A(t)]S^0 \quad (3)$$

By utilizing $S^*(t) \doteq S(0) \cdot e^{-k^*t}$ where k^* is the corrected fractional synthesis rate, then eqn. (3) could be solved for k^* :

$$k^* = \frac{1}{t} \cdot \ln \left(\frac{S(0)}{S^*(t)} \right) \quad (4)$$

The corrected fractional degradation rate k^* will be given by:

$$k^* = \frac{1}{t} \cdot \ln \left(\frac{S(0) \cdot A(0)}{S^*(t) \cdot A(t)} \right) \quad (5)$$

Results and discussion

Evidence for the existence of re-utilization of pyrimidine nucleotides

In order to ascertain whether RNA-degradation products are re-utilized for the synthesis of new RNA, we monitored, with time after partial hepatectomy, the decay of specific radioactivity of (a) total acid-soluble uridine nucleotides pool, (b) uridine and cytidine components of total cellular RNA, as it was previously shown that rRNA comprises a constant 85% of cellular RNA during the course of liver regeneration (Nikolov *et al.*, 1983), and (c) total rRNA and its pyrimidine nucleotides (Fig. 1).

The rapid loss of radioactivity of the hydrolysed acid-soluble UMP pool during the first 3 days of liver regeneration reflects the quick expansion of the pool of free nucleotides, necessary for the intensive synthesis of RNA on the one hand and the increased flow of radioactivity to the CTP pool and to newly synthesized RNA on the other. The rate of expansion of the UTP pool during the proliferative phase of liver regeneration (days 1–3) was 0.0423 h^{-1} , corresponding to a synthesis rate of 102%/day (Table 1). An interesting observation, consistent with the idea of re-utilization of pyrimidine nucleotides, originating from RNA degradation, was the increase in the specific radioactivity of the whole-cell UMP pool from day 3 until day 5. This fact could be explained with the onset of more intense rRNA degradation after day 3, as has been shown (Nikolov *et al.*, 1983), and/or shrinkage of the acid-soluble UMP pool after that day. From day 5 on the specific radioactivities of the acid-

Fig. 1. Time course of the decay of specific radioactivities of the acid-soluble UMP pool and of pyrimidine nucleotides of total cellular and nuclear RNA in regenerating rat liver

Rats were injected intraperitoneally with $200 \mu\text{Ci}$ of $[2\text{-}^{14}\text{C}]\text{orotate}$ per rat 5 days before partial hepatectomy and killed in groups of three at the times indicated after operation. The isolation of whole-cell pool and of cellular and nuclear RNA, the separation of their pyrimidine nucleotides and the determination of specific radioactivities were performed as described in the Experimental section. Mean values for three rats are plotted at each time point, and the s.d. values are generally less than 10% of the mean in each case. Left-hand scale: ▲—▲, whole-cell uridine nucleotides pool; ○—○, RNA-UMP and □—□, RNA-CMP; ●—●, nRNA-UMP; ■—■, nRNA-CMP. Right-hand scale: Δ—Δ, total cellular RNA hydrolysate. The inset shows a plot of the relative increase of rRNA amount in the regenerating liver $[A(t)/A(0)]$ against time after partial hepatectomy. The rRNA gain for each time point is calculated as described in the Experimental section and is mean $\pm$ s.d., $n=3$. The rRNA content of the initial remnant $[A(0)]$ is taken as 1.

soluble UMP and total RNA hydrolysate decayed in parallel (see Table 1). In our previous paper we pointed out that after the fifth day of liver regeneration the liver cell is returning to its normal state (Nikolov *et al.*, 1983), and that is most probably why the size of the pool of free uridine nucleotides does not change any more. The constancy of the pool size is reflected in the exponential decay of the acid-soluble UMP specific radioactivity parallel to that of RNA hydrolysate. The determination of turnover rate

Table 1. Turnover data for the pyrimidine components of cellular and nuclear RNA and acid-soluble UMP pool during rat liver regeneration

k' and k are the fractional synthesis and degradation rates of RNA, calculated respectively by the equations $S(t)/S(0) = e^{-k't}$ and $S(t)A(t)/S(0)A(0) = e^{-kt}$, where $S(0)$ and $S(t)$ (d.p.m./ μ g of RNA) are the specific radioactivities of RNA in the excised lobes and at time t after partial hepatectomy, and $A(0)$ and $A(t)$ are the calculated amounts of RNA in the initial remnant and in the regenerating liver respectively (their values are taken from Nikolov & Dabeva, 1983). k' and k are calculated on a day-to-day basis. T' is the synthesis time for the respective pool and $T_{\frac{1}{2}}$ is the half-life. The values for the proliferative and post-proliferative phases are means for days 1–3 and days 6–12 respectively. Each value is a mean $\pm$ S.D. for nine animals. The corrected values of RNA-UMP are calculated according to eqns. (4) and (5) (Experimental section). Further abbreviations used: RNA-UMPCor., the corrected value of RNA-UMP on the basis of the acid-soluble UMP pool; RNA-UMPCorN., the corrected value of RNA-UMP on the basis of nRNA-UMP.

Synthesis	Proliferative phase		Post-proliferative phase	
	k' (h^{-1})	T' (h)	k' (h^{-1})	T' (h)
RNA hydrolysate	0.0144 ± 0.0027	69.4	0.0071 ± 0.0010	140.8
RNA-CMP	0.0125 ± 0.0013	80.0	0.0068 ± 0.0009	147.1
RNA-UMP	0.0161 ± 0.0023	62.1	0.0087 ± 0.0019	114.9
nRNA	0.0200 ± 0.0024	50.0	0.0062 ± 0.0009	161.3
nRNA-CMP	0.0198 ± 0.0012	50.5	0.0051 ± 0.0011	196.1
nRNA-UMP	0.0202 ± 0.0028	49.5	0.0069 ± 0.0005	144.9
Acid-soluble UMP	0.0423 ± 0.0014	23.6	0.0070 ± 0.0023	142.9
RNA-UMPCor.	0.0173 ± 0.0018	57.8	0.0090 ± 0.0027	111.1
RNA-UMPCorN.	0.0202 ± 0.0026	49.5	0.0093 ± 0.0028	107.5
Degradation	k (h^{-1})	$T_{\frac{1}{2}}$ (h)	k (h^{-1})	$T_{\frac{1}{2}}$ (h)
RNA hydrolysate	0.0022 ± 0.0020	315.1	0.0059 ± 0.0010	117.1
RNA-UMP	0.0025 ± 0.0016	277.3	0.0076 ± 0.0006	91.2
RNA-CMP	0.0019 ± 0.0013	364.8	0.0058 ± 0.0013	119.5
RNA-UMPCor.	0.0029 ± 0.0019	239.0	0.0081 ± 0.0009	85.6
RNA-UMPCorN.	0.0034 ± 0.0021	203.9	0.0087 ± 0.0011	79.7

for the whole-cell pool of uridine nucleotides during the post-proliferative phase showed a half-life of 99 h (Table 1). The corresponding value in the proliferative phase was not calculated because the pool size of free UTP was not measured.

The decay of specific radioactivities of the whole-cell pool of uridine nucleotides and of nRNA-UMP during the proliferative phase followed a significantly different but not independent pattern. The expansion of the acid-soluble UMP pool was accompanied by a decrease in the specific radioactivity of nRNA-UMP, the latter being more gradual (fractional rate of synthesis $k' = 0.0423$ compared with 0.0202) (Table 1). During the post-proliferative phase, after day 6, the two specific radioactivities equilibrated and decayed in parallel thereafter ($k' = 0.0070$ and 0.0069 respectively). This is in agreement with the results obtained by Blobel & Potter (1968), who pointed to the parallel decay rates of labelled nRNA and of the acid-soluble pool in normal rat liver as a suggestion for the existence of nucleotide re-utilization. The specific radioactivity of the whole-cell UMP pool was lower than that of the uridine component of nRNA during the whole

period studied after partial hepatectomy. The ratio of specific radioactivity of nRNA-UMP to that of the acid-soluble UMP rose sharply during the first 2 days after partial hepatectomy, then decreased gradually and remained constant during the post-proliferative phase.

Another fact consistent with the idea of re-utilization of pyrimidine nucleotides originating from cytoplasmic RNA degradation is the increase in the specific radioactivities of both uridine and cytidine components of nRNA on days 4 and 5 after partial hepatectomy, following to a lesser extent the pattern of the whole-cell pool of uridine nucleotides (Fig. 1). Such an elevation can be observed even in the specific radioactivities of total cellular RNA and its pyrimidine nucleotides.

As shown in Fig. 1, the decay of specific radioactivities in the uridine and cytidine components of cellular RNA was biphasic, with a fast phase and a slow phase, corresponding to the proliferative and post-proliferative phases of liver regeneration. The labelling of RNA-UMP was higher than that of RNA-CMP over the entire period studied after partial hepatectomy. This is reflected also in the ratio of specific radioactivities

of RNA-CMP and RNA-UMP (see below). The decrease of the specific radioactivity of RNA-UMP was faster than that of RNA-CMP during both phases of liver regeneration (Table 1), showing that RNA-CMP apparently turned over more slowly than RNA-UMP. The lower turnover rate of RNA-CMP during liver regeneration points to the higher extent of re-utilization of cytidine nucleotides for the synthesis of new RNA. This is the consequence of the flow of the cytidine components of degraded cytoplasmic RNA into a pool of free cytidine nucleotides in the cytoplasm that is 5–6-fold smaller than the respective pool of uridine nucleotides (Bucher & Swaffield, 1966), and hence the higher specific radioactivity of CTP that is re-utilized into RNA compared with that of UTP.

A third line of evidence in support for the recycling of preformed pyrimidines is the increase in the C/U ratio (specific radioactivity of CMP to that of UMP) in cytoplasmic and in nuclear RNA in the course of rat liver regeneration (Fig. 2). The rise in the C/U ratio during the first day followed by a decline during the second day after partial hepatectomy, observed in the behaviour of both RNA species, may reflect the successive expansion of UTP and CTP pools during liver regeneration, in agreement with previous reports (see Bucher & Malt, 1971). The C/U ratio in cytoplasmic RNA was 0.57 in the resected lobes; this then increased gradually as regeneration progressed and reached a value of 1.0 about 12 days after partial hepatectomy. This rather slow equilibration of the two specific radioactivities in total RNA is most probably a consequence of the decreased degradation of RNA during the first days of the regenerative process. The C/U ratio was all the time higher in the nuclei than in the cytoplasm. In nRNA it reached 1.0 on approximately day 5 after operation, increasing further from that day on. Since the specific radioactivities of the uridine and cytidine nucleotides in the acid-soluble pool of rat liver cells are essentially identical and they turn over at the same rate (the C/U ratio of the free nucleotides in the cytoplasm has a constant value of 0.9 from day 3 on) (Fig. 2), the observed increases in the C/U ratio in nuclear and cytoplasmic RNA suggest also the existence of a partial compartmentalization of nuclear and cytoplasmic pyrimidine ribonucleotide pools. If the whole-cell ribonucleotide pool is the source of precursors for nRNA synthesis, then the C/U ratio in the nRNA species will reflect the C/U ratio in the pool. The above data suggest that this is not the case in rat liver cells.

The specific radioactivity of the whole-cell UMP pool was lower than that of nRNA-UMP during the whole period studied after partial

Fig. 2. Kinetics of increase of the C/U ratio (specific radioactivity of CMP to that of UMP) as function of time after partial hepatectomy

The specific radioactivities for the experimental points were determined as described in the Experimental section. Points denote means for three rats, and the s.d. values are generally less than 15% of the mean in each case. ■—■, Total cellular RNA; ●—●, nRNA; △—△, whole-cell pool of uridine nucleotides.

hepatectomy. The ratio of specific radioactivity of nRNA-UMP to that of acid-soluble UMP rose sharply after the operation, reaching a value of 6.0 on day 2 of liver regeneration, then decreased gradually and attained a constant value of 1.8 during the post-proliferative phase (Fig. 1). It should be pointed out that it is impossible to obtain absolutely pure nuclei, even after Triton X-100 treatment (Dabeva *et al.*, 1977) as in our experimental procedure. Nevertheless, the difference in the ratio of the specific radioactivity of nRNA-UMP to that of the whole-cell UMP pool during both phases of liver regeneration shows that the higher specific radioactivity of nRNA-UMP could not be due solely to cytoplasmic contamination of the nuclear fraction, because it is not logical to assume different extents of contamination during the proliferative and post-proliferative phases. This observation shows that the specific radioactivity of pyrimidine precursors used for nRNA synthesis is higher than the specific radioactivity of the whole-cell pool of pyrimidine nucleotides. This suggests that nRNA as a whole could not be synthesized from the cytoplasmic pool of acid-soluble nucleotides as the specific radioactivity of the nucleotides in nRNA species should be a direct reflection of the specific radioactivity of the free nucleotides from which nRNA is synthesized.

Our approach in which the two major classes of nRNA are not separately analysed does not rule out the possibility that only nucleolar RNA is synthesized from a nuclear precursor pool whereas the precursors for hnRNA formation are supplied from the cytoplasmic pool (Wiegers *et al.*, 1976; Birch & Turnock, 1982).

The higher specific radioactivity of nRNA-UMP in comparison with that of the whole-cell UMP pool and the established equilibrium

between the two pools during the post-proliferative phase (an equilibrium in which the specific radioactivity of nRNA-UMP is 1.6–2-fold higher) are further suggestive evidence for the existence of compartmentalized nucleotide pools, with a one-way flow from the cytoplasmic pool to the nuclear one. The precursors used for the synthesis of nRNA are supplied from the cytoplasmic pool of free nucleotides and from the degradation of fast-turning-over hnRNA and/or pre-rRNA.

Previous studies on uridine metabolism in cultured cells (reviewed by Hauschka, 1973) have shown that UTP arises from both synthesis *de novo* and the phosphorylation of UMP derived from the degradation of RNA molecules within the cell. For nucleotides to be re-utilized, they have to be in a 5'-phosphorylated form. The existence of both nuclear and cytoplasmic enzymes with 5'-exonuclease activity in animal cells has been reported (Harris, 1963; Sporn *et al.*, 1969; Kumagai *et al.*, 1979).

Extent of re-utilization of pyrimidine nucleotides

The re-utilization of labelled RNA degradation products into newly synthesized RNA results in a slower decay of the specific radioactivity in RNA than would be expected from the true turnover. Here we tried to assess the extent of recycling of radioactive precursor on the basis of decay of

radioactivity in RNA-UMP and the changes in the specific radioactivity of the whole-cell UMP pool, according to eqns. (4) and (5) (Experimental section). The corrected values for the rates of synthesis and degradation of RNA-UMP were higher than the uncorrected ones during both phases of liver regeneration (Table 1). Comparing the corrected and uncorrected values for the synthetic rate of RNA-UMP during the proliferative phase, the extent of re-utilization of uridine nucleotides was found to be 7.0%/day (Table 2), because of the relatively high specific radioactivity of the total acid-soluble UMP pool. As a consequence of the endogenous chase occurring during the first days after partial hepatectomy and leading to the rapid decrease in the specific radioactivity of the whole-cell uridine nucleotides pool, the correction for recycling of the uridine components was minimal (3.2%/day) in the post-proliferative phase. If turnover measurements were based on the decay of radioactivity of total RNA hydrolysate, then the correction for pyrimidine derivatives re-utilization would be 16.6%/day and 21.3%/day for the two phases of liver regeneration respectively, because of the considerably higher re-utilization of the cytidine nucleotides. The extent of re-utilization of CMP, calculated as a difference between the rate of recycling of total pyrimidine components of RNA and that of the uridine nucleotides, was more pronounced in the second phase (9.6%/day and 18.1%/day for the two phases respectively).

The endogenous chase occurring at the beginning of liver regeneration and diluting the labelled intermediates in the process of recycling could explain why in this and previous studies (Nikolov *et al.*, 1983; Nikolov & Dabeva, 1983) we obtained shorter half-lives for rRNA in the post-proliferative phase in comparison with other authors for normal rat liver (Loeb *et al.*, 1965; Hirsch & Hiatt, 1966; Wilson & Hoagland, 1967; Nordgren & Stenram, 1972; Vacha & Seifert, 1975; Goodlad & Ma, 1975), even though our measurements were based on specific radioactivities in total rRNA hydrolysate. These results show that the half-lives could be calculated more accurately from the decay of specific radioactivity of RNA-UMP and that there might be a 21% or more overestimate in the turnover rate of rRNA in normal rat liver because of the existing re-utilization of pyrimidine components in RNA degradation products.

If we assume that the pool that serves the synthesis of nRNA is the one in the nuclei, with a specific radioactivity equal to that of nRNA, then an analogous correction of RNA-UMP on the basis of specific radioactivity of nRNA-UMP would yield values for rRNA turnover that are much higher than those obtained after a correc-

Table 2. *Extent of re-utilization of pyrimidine components of RNA during rat liver regeneration*

The re-utilization of pyrimidine nucleotides is calculated from the differences in the synthetic rates of RNA hydrolysate, RNA-UMP and the corrected values of RNA-UMP on the basis of acid-soluble UMP pool (column a) and on the basis of nRNA-UMP (column b). Abbreviations used: RNA-(UMP+CMP), pyrimidine components of total cellular RNA; RNA-UMPcor., the corrected value of RNA-UMP on the basis of the acid-soluble UMP pool; RNA-UMPcorN., the corrected value of RNA-UMP on the basis of nRNA-UMP.

	Synthesis rate (%/day)	Re-utilization (%/day)	
		a	b
Proliferative			
RNA-(UMP+CMP)	34.6	16.6	28.7
RNA-UMP	38.6	7.0	20.4
RNA-CMP		9.6	8.3
RNA-UMPcor.	41.5		
RNA-UMPcorN.	48.5		
Post-proliferative			
RNA-(UMP+CMP)	17.0	21.3	23.8
RNA-UMP	20.9	3.2	6.3
RNA-CMP		18.1	17.5
RNA-UMPcor.	21.6		
RNA-UMPcorN.	22.3		

tion, based on the whole-cell pool of uridine nucleotides (Tables 1 and 2). The difference is greater in the proliferative phase (fractional rate of degradation $k=0.0034\text{h}^{-1}$ compared with 0.0029h^{-1}) than in the post-proliferative phase (the respective values are 0.0087h^{-1} and 0.0081h^{-1}) (Table 1).

However, all the above considerations would not change the main conclusion of our previous studies that the turnover of rRNA is markedly slower during the first 3 days of rat liver regeneration (Nikolov *et al.*, 1983; Nikolov & Dabeva, 1983).

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