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Pyrimidine metabolism and sugar nucleotide synthesis in rat liver

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With radioactive precursors, the labelling kinetics of the soluble pyrimidine nucleotides and of RNA were measured in rat liver to determine the contribution of the metabolic flows through synthesis *de novo* and the salvage pathway. To separate and quantify all pyrimidine nucleotides, an h.p.l.c. technique was developed using anion-exchange chromatography and reversed-phase chromatography. The concentrations of cytidine nucleotides were in the range of 30–45 nmol/g wet weight, and the concentrations of the uridine phosphates and of the UDP-sugars were approx. 6 and 20 times higher respectively. After a single injection of [^{14}C]orotic acid and of [^{3}H]cytidine, the specific radioactivities were determined as a function of time. The $^{14}C/^{3}H$ ratio was calculated and gave a good indication of the involvement of the different flows. It could be concluded that UTP derived from synthesis *de novo* and from the salvage pathway is not completely mixed before being utilized. The flow of the salvage pathway is relatively more directed to RNA synthesis in the nucleus and that of synthesis *de novo* to cytoplasmic processes. For CTP it could also be concluded that the flow of the salvage pathway was relatively more directed to RNA synthesis in the nucleus. Because of the nuclear localization of the enzyme CMP-NeuAc (*N*-acetylneuraminate) synthase, special attention was paid to CMP-NeuAc. However, a conclusion about a location about the synthesis of CMP-NeuAc could not unequivocally be drawn, because of the small differences in $^{14}C/^{3}H$ ratio and the different values for the CDP-lipids.

INTRODUCTION

One of the most important co- and post-translational modifications of proteins is glycosylation. This is a complex process starting in the lumen of the rough endoplasmic reticulum and ending in the Golgi system, during which specific glycosyltransferases transfer sugars from the donor nucleotide sugars on to proteins and lipids (for a review, see [1]). Regulation occurs by compartmentation of the enzyme systems, activities of the specific glycosyltransferases, transport rates of the acceptor molecules through the lumen of the endoplasmic reticulum and the availability of the various cofactors, e.g. nucleotide sugars.

During our investigation of the metabolism of sialic acid, a negatively charged nine-carbon sugar which is transferred on to oligosaccharide chains in the Golgi system, we became interested in the regulation of the glycosylation by the availability of nucleotide sugars. It is known that the sialic acid-activating enzyme, CMP-NeuAc (*N*-acetylneuraminate) synthase (EC 2.7.7.43) is located in the nucleus [2–7], whereas all other known sugar-activating enzymes are located in the cytosol [6,7]. Until now the reason for this peculiar localization is unknown, although it is questionable whether the enzyme is functionally active in the nucleus [8]. In the cell, nucleoside triphosphates (NTP) are synthesized by a '*de novo*' and a salvage pathway. Intracellular compartmentation of NTP pools may be the result of separate flows of nucleotide precursors through those pathways. Many studies concerning RNA synthesis have reported compartmentation of UTP pools (for reviews, see [9–11]). Such compartmentation might also be important for the regulation of the synthesis of the various uridine nucleotide sugars. As CTP is also synthesized by a salvage pathway and from UTP by synthesis *de novo*, compartmentation of the CTP pool can exist, too; however, this has never been reported. To investigate the metabolism of nucleotide sugars, especially CMP-NeuAc and the CDP-lipids (CDP-ethanolamine and CDP-choline) with regard to various nucleotide flows, we developed an h.p.l.c. system for a complete separation of the pyrimidine nucleotides. With this system we have measured the labelling kinetics of the various pyrimidine nucleotides in rat liver after administration of [6-^{14}C]orotic acid and [5-^{3}H]cytidine. These compounds are incorporated by the '*de novo*' and the salvage pathway respectively into pyrimidine nucleotides [11]. In order to discriminate between the various UTP and CTP flows to several areas of cellular metabolism, we used the nucleotides 2′/3′-CMP and 2′/3′-UMP derived from RNA as representatives of the nuclear compartment, and all UDP-sugars and CDP-lipids were considered to be representatives of the cytosolic/microsomal compartment. In this paper we describe the results of our experiments *in vivo*. Part of this work has been presented in preliminary form [12].

MATERIALS AND METHODS

Chemicals

[6-^{14}C]Orotic acid (sp. radioactivity 61 Ci/mol) and [5-^{3}H]uridine (sp. radioactivity 29000 Ci/mol) were products of The Radiochemical Centre, Amersham, Bucks., U.K.; [5-^{3}H]cytidine (sp. radioactivity 26000 Ci/mol) was a product of The Radiochemical Centre or Moravek Biochemicals, Brea, CA, U.S.A. During our experiments The Radiochemical Centre ceased supplying cytidine. 1,1,2-Trichlorotrifluoroethane, tri-n-octylamine and per-

Abbreviations used: NTP, nucleoside triphosphates; PCA, perchloric acid; CXP-compounds, CMP-*N*-acetylneuraminate + CDP-ethanolamine + CDP-choline; NeuAc, *N*-acetylneuraminate.

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chloric acid (PCA) were obtained from Merck, Darmstadt, Germany.

The nucleotides used as chromatographic standards were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A., or Boehringer Mannheim, Mannheim, Germany. All other chemicals were obtained from commercial sources and were of the highest quality available.

Animals

Male Wistar rats of age 2–3 months (weighing 150–200 g) were kept on a light/dark schedule and fed for 2 h each day as described previously [13]. The rats were starved for 24 h before use. Three series of rats (seven to nine each) were injected (intravenously into the tail vein) with 0.5 ml of phosphate-buffered saline (0.05 M-phosphate/0.15 M-NaCl), pH 7.4, containing 15 μCi of [^{14}C]orotic acid and 100 μCi of [^{3}H]uridine or 100 μCi of [^{3}H]cytidine. Rats were killed by immersing the whole animal in liquid N_2 and stored at -40 °C until use. Livers were removed in a frozen state. The frozen livers were weighed and ground in a mortar under liquid N_2 and extracted with 0.4 M-PCA or ethanol (75%, v/v) as described below.

The ATP/ADP ratio of the PCA extract of the livers was 0.8–1.0. A ratio of 0.5–0.7 was found for livers obtained immediately after killing by cervical dislocation and storage at -40 °C (results not shown).

Extraction of soluble nucleotides

Samples of ground livers (approx. 0.5 g) were homogenized in a 15 ml Potter–Elvehjem homogenizer tube with a Teflon pestle and extracted with 5 ml of ice-cold 0.4 M-PCA. After centrifugation the pellet was washed twice with half of the original volume of 0.2 M-PCA. From the combined supernatants the PCA was removed by adding 2 vol. of a mixture of tri-n-octylamine and trichlorotrifluoroethane (1:4, v/v) as described by Khym [14]. After centrifugation the organic phase was removed and washed twice with 1 vol. of twice-distilled water. The aqueous phases were combined and freeze-dried. The residue was redissolved in twice-distilled water giving a final concentration of 1 g liver wet weight/ml of water (PCA extract). A separate extraction with ethanol was performed to give a maximal yield of labile nucleotides, especially CMP-sialic acid. Approx. 0.5 g of liver was extracted with ethanol (75%, v/v) as described above for PCA. The extract and two washings were combined and evaporated. Lipids were then removed by extracting the residue with 15 ml of water/methanol/chloroform (3:5:10, by vol.). After centrifugation the upper phase (approx. 6.0 ml) was withdrawn. The chloroform containing lower phase was washed once with 6.0 ml of methanol/water (1:1, v/v). The combined upper phases were evaporated and redissolved in twice-distilled water to give a final concentration of 1 g liver wet weight/ml of water (ethanol extract).

Preparation of RNA hydrolysate

The pellet obtained after PCA extraction was incubated with 5 ml of 0.2 M-KOH at 37 °C for 18 h to hydrolyse specifically the RNA and then mixed at 0 °C with 250 μl of 10 M-PCA. After 15 min the mixture was centrifuged (3500 *g*, 10 min) and the supernatant removed. The pellet was washed twice with 2.5 ml of 0.2 M-PCA and combined with the first supernatant. From this total RNA hydrolysate the excess PCA was removed with 2 vol. of tri-n-octylamine/trichlorotrifluoroethane mixture as described above. The final residue was redissolved in twice-distilled water, giving a final concentration of 1 g liver wet weight/ml of water.

Chromatographic procedures

Chromatography was performed on a Perkin–Elmer series 2 liquid chromatograph equipped with a Rheodyne injection valve. The compounds were detected with a Perkin–Elmer LC-75 variable-wavelength detector, operating at 262 nm, combined with a Spectra Physics SP 4100 computing integrator. The first separation of the soluble nucleotides was done on a Whatman Partisil-10-SAX column (4 mm × 250 mm, particle size 10 μm) as described by Zakaria & Brown [15]. Separation was achieved with a phosphate buffer concentration gradient; the low-concentration buffer (A) was 5 mM-H_3PO_4/KH_2PO_4, pH 4.0, and the high concentration buffer (B) was 0.5 M-KH_2PO_4/K_2HPO_4, pH 4.5. A three-step linear gradient was used, starting with buffer A: an increase in B first at 0.3%/min for 30 min, followed by 2.5%/min for 15 min and finally at 5%/min for 10 min until 99% B was reached, which conditions were maintained until the end of the run (total of 70 min). The flow rate was 1.5 ml/min. Before starting a new run the column was equilibrated for 25 min with 100% A. Combinations of the first 18 min fractions were taken and freeze-dried: 0–4.5 min, 4.5–9 min, 9–13.5 min and 13.5–18 min. The last three combined fractions were rechromatographed on a Lichrosorb RP-18 column (Merck; 4 mm × 250 mm; particle size 4 μm). Starting with 0.1 M-H_3PO_4/KH_2PO_4 buffer, pH 4.0, the separation was accomplished with a linear gradient of the same solution, containing 20% (v/v) methanol; the increase was 2.5% of the methanol solution/min for 30 min. The flow rate was 0.5 ml/min. Before injection of the next sample the column was equilibrated for 15 min with the starting solution. Chromatography of the RNA hydrolysate was also done on the Lichrosorb RP-18 column under the same conditions as those described above. From the integrated areas of the various peaks and standards the concentrations of the various nucleotides were calculated. For measurement of the radioactivity, 0.5 min fractions were collected and counted in a Berthold BF 8000 liquid-scintillation counter equipped with an automatic external standard for calculation of the d.p.m.

RESULTS

Extraction of the liver with PCA caused 50–75% hydrolysis of CMP-NeuAc, whereas hydrolysis of the other nucleotides was less than 5%. From earlier experiments it was known that, if ethanol was used for the extraction of the nucleotide sugars, minimal hydrolysis of the labile CMP-NeuAc occurred [16]. However, it appeared that, after extraction of liver tissue with ethanol, a low recovery of other nucleotides was obtained, especially the di- and tri-phosphates. This was probably due to a strong adsorption to precipitating molecules, since after extraction of the precipitate with PCA, the di- and tri-phosphates could still be recovered without any notable hydrolysis (results not shown). Because we were interested in all pyrimidine nucleotide derivatives, including CMP-NeuAc, we decided to perform tissue extraction with ethanol as well as with PCA. By com-

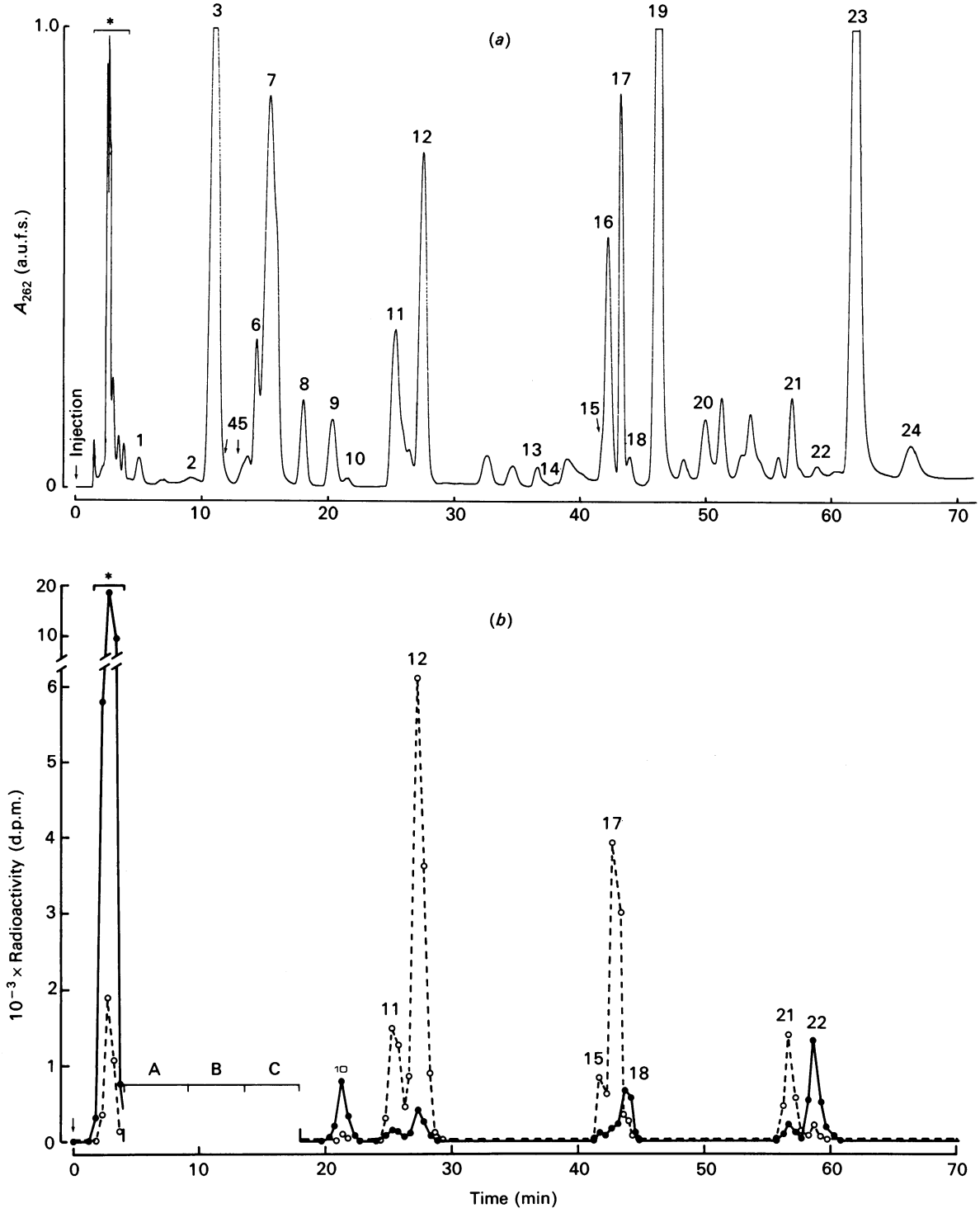

Fig. 1. H.p.l.c. pattern of nucleotides extracted with PCA from rat liver separated on Partisil-10-SAX

Conditions were as described in the Materials and methods section. (*a*) Profile of u.v.-absorbing compounds, recorded at 262 nm and expressed in arbitrarily units full scale (a.u.f.s.). (*b*) Profile of ^{14}C- (○) and ^{3}H-labelled (●) pyrimidine nucleotide compounds 20 min after injection of a mixture of 15 μCi of [6-^{14}C]orotic acid and 100 μCi of [5-^{3}H]cytidine. Compounds identified: *, bases and nucleosides; 1, CDP-ethanolamine; 2, CMP; 3, NAD^+; 4, CDP-choline; 5, orotic acid; 6, UMP; 7, AMP; 8, IMP; 9, GMP; 10, CMP-NeuAc; 11, UDP-*N*-acetylglucosamine (*N*-acetylgalactosamine); 12, UDP-glucose (galactose); 13, GDP-mannose; 14, GDP-fucose; 15, UDP; 16, $NADP^+$; 17, UDP-glucuronic acid; 18, CDP; 19, ADP; 20, GDP; 21, UTP; 22, CTP; 23, ATP; 24, GTP. Fractions A, B and C were taken for rechromatography (see Fig. 2).

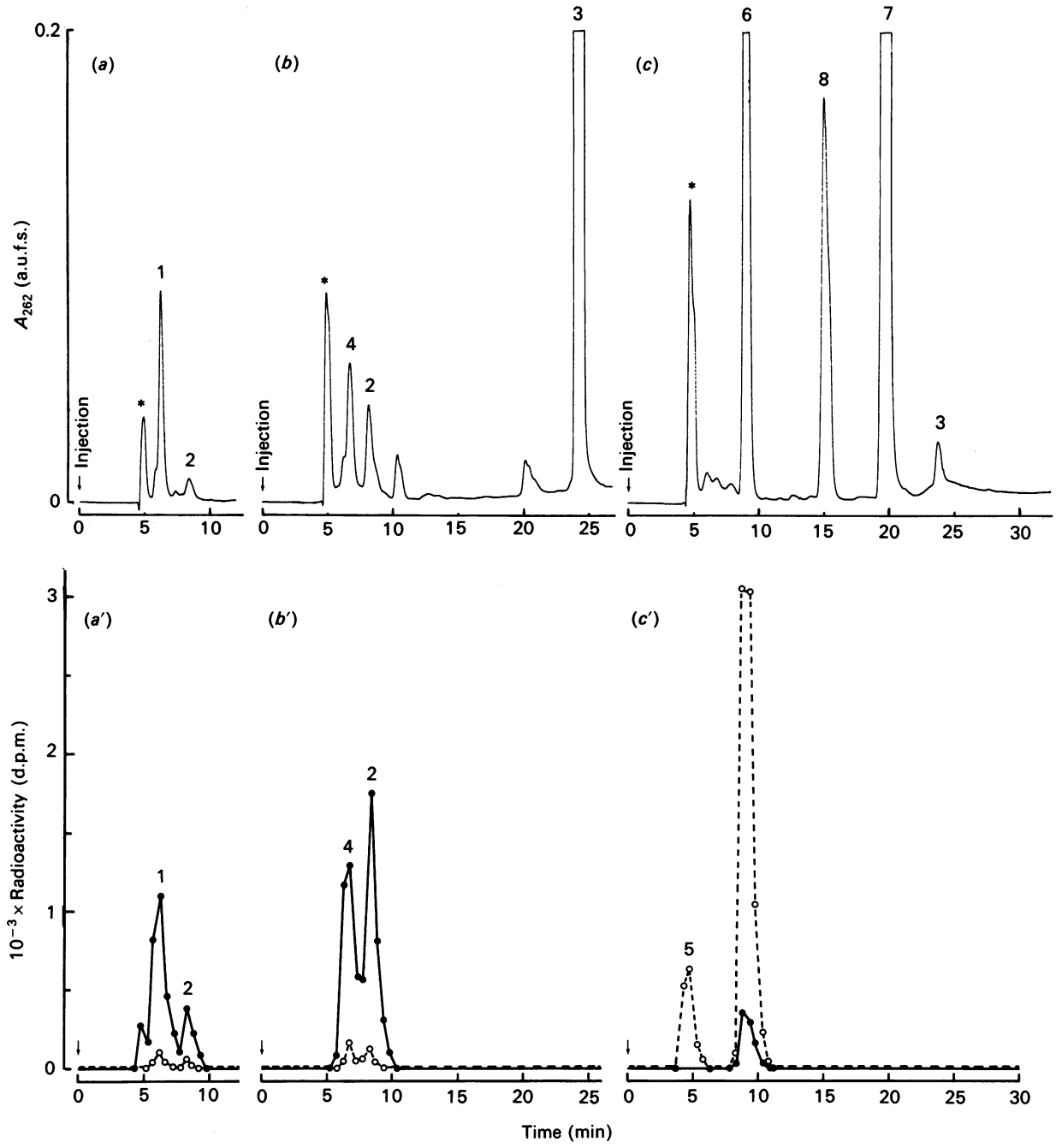

Fig. 2. Reversed-phase h.p.l.c. separation of some nucleotides from rat liver on Lichrosorb RP-18

Three different combined fractions obtained after a first separation of a PCA extract on Partisil-10-SAX (A, B and C; see Fig. 1) were rechromatographed as described in the Materials and methods section. Profiles of the u.v.-absorbing compounds, recorded at 262 nm, of the combined fractions from 4.5–9.0 min (*a*), 9.0–13.5 min (*b*) and 13.5–18.0 min (*c*) and the corresponding profiles (*a'*, *b'* and *c'*) of the ^{14}C- (○) and ^{3}H-labelled (●) pyrimidine nucleotide compounds, 20 min after injection of a mixture of 15 μCi of [6-^{14}C]orotic acid and 100 μCi of [5-^{3}H]cytidine. The numbers identifying the peaks correspond to those in Fig. 1. *, Solvent front containing buffer salts from Partisil-SAX.

bination of the results obtained after the analysis of both extracts we were able to determine almost all pyrimidine nucleotides. During preparation of the sample for h.p.l.c. injection, minimal losses of material occurred. More than 97% of the nucleotides was recovered, as measured with labelled standards. On the basis of the work of Zakaria & Brown [15] the first separation of the soluble nucleotides was done on a strong-anion-exchange Partisil-SAX column with phosphate buffer as eluent. After testing different combinations of concentrations of phosphate buffer, flow rate and time, the best results were obtained with the method described in the Materials and methods section. The u.v. chromatogram of the anion-exchange separation of the soluble nucleotides

Table 1. Concentration of pyrimidine ribonucleotides in rat liver and their labelling after injection of radioactive precursors

For the extraction procedure and separation of the compounds, see the Materials and methods section. The concentrations were calculated from the integrated peak areas, which were compared with those of known quantities of standards. Labelling was achieved by the injection of 15 μCi of [6-^{14}C]orotic acid and 100 μCi of [5-^{3}H]cytidine simultaneously. The ^{14}C/^{3}H ratios were calculated from the total disintegrations/peak or from some best-separated fractions (CDP). Most values are expressed as the mean ± S.D. of all results obtained with PCA extraction as well as ethanol extraction. The numbers of rats used are given in parentheses. Abbrevations: GlcA, glucuronic acid; HexNAc, *N*-acetylhexosamine; –, too unreliable to measure.

Compound	Amount (nmol/g wet wt.) (n = 12)	Specific radioactivity (d.p.m./nmol)				^{14}C/^{3}H ratio
		^{14}C			^{3}H	
Time (min)...		5 (2)	10 (3)	20 (3)	10 (3)	10 (3)
CDP-ethanolamine	41 ± 4	37, 39	49 ± 10	40 ± 2	1175 ± 432	0.043 ± 0.007
CDP-choline	39 ± 6	87, 72	75 ± 6	60 ± 12	1245 ± 364	0.063 ± 0.012
CMP-NeuAc*	32 ± 8	23, 21	42 ± 13	45 ± 9	1224 ± 591	0.036 ± 0.006
CMP	32 ± 6	43	84 ± 8	88 ± 11	1707 ± 621	0.054 ± 0.019
CDP	–	–	–	–	–	0.065 ± 0.013
CTP†	44 ± 9	66, 63	77 ± 23	73 ± 19	1520 ± 730	0.051 ± 0.011
2′-+3′-CMP (RNA)	2577 ± 353	0.22, 0.21	0.56 ± 0.13	0.89 ± 0.23	20.8 ± 7.8	0.028 ± 0.005
UDP-HexNAc	577 ± 110	78, 111	124 ± 45	159 ± 59	6.7 ± 2.1	18.4 ± 2.7
UDP-Hex	869 ± 76	286, 325	392 ± 67	281 ± 54	14.8 ± 2.1	26.7 ± 4.5
UDP-GlcA	754 ± 86	138, 82	190 ± 56	211 ± 73	6.8 ± 2.4	29.0 ± 6.8
UMP	205 ± 38	609, 613	485 ± 106	345 ± 51	26.2 ± 10.4	13.5 ± 0.8
UDP	–	–	–	–	–	21.4 ± 3.5
UTP†	212 ± 21	603, 587	469 ± 95	297 ± 45	30.4 ± 5.3	15.2 ± 2.1
2′-+3′-UMP (RNA)	1395 ± 179	8.2, 9.0	10.8 ± 2.9	10.6 ± 1.0	1.2 ± 0.6	10.2 ± 5.3

* Results of the ethanol extraction only.
† Results of the PCA extraction only.

obtained after PCA extraction is shown in Fig. 1(*a*). Most nucleotides could be separated within a reasonable time. In spite of the very low phosphate gradient (0.3%/min) in the beginning of the run, it was not possible to obtain a complete separation of the fast-eluted pyrimidine derivatives, namely CMP, UMP, CDP-ethanolamine and CDP-choline. Rechromatography of combined fractions on a reversed-phase column resulted in a complete separation of these pyrimidine nucleotides from the high-u.v.-absorbing peaks of NAD^+ and AMP and from [^{14}C]orotic acid and catabolites (Figs. 2*a*, 2*b* and 2*c*). The combined fractions from the Partisil-SAX column could be freeze-dried and rechromatographed without desalting, because they contained low concentrations of phosphate. We also collected fractions containing higher phosphate concentrations, e.g. between 40 and 45 min, containing UDP, $NADP^+$ and UDP-GlcA. These fractions could could not be rechromatographed without being desalted. However, desalting on small Sep-Pak C_{18} columns (Waters Associates) resulted in great losses of the nucleotides and/or introduced interfering u.v.-absorbing substances (results not shown). The levels of the pyrimidine nucleotides in the liver are summarized in the first column of Table 1. For UDP and CDP, no reliable concentration could be determined, because in our h.p.l.c. system they were co-eluted with $NADP^+$ and UDP-GlcA respectively. A change in the chromatographic conditions to separate these compounds resulted in incomplete separation of nucleotides in other parts of the run or an undesirable long run time. The 2′- and 3′-monophosphate nucleotides, obtained from RNA after alkaline hydrolysis, were easily separated on the Lichrosorb column (results not shown). Elution times for 3′-CMP, 2′-CMP, 3′-UMP and 2′-UMP were 11.5, 12.2, 14.0 and 15.7 min respectively under the conditions as described.

Before starting our incorporation studies, [^{3}H]uridine, [^{3}H]cytidine and [^{14}C]orotic acid were tested for their uptake and metabolism by the liver. Within 30 min after injection of [^{3}H]uridine we could find hardly any incorporation of label into the soluble nucleotides and RNA. This is in agreement with the results of others [17–20] and attributable to high uridine catabolism in the liver [17,21,22]. As reported by Moyer *et al.* [20] and Dahnke [23], [^{3}H]cytidine was much better incorporated into soluble nucleotides and RNA. After 5 min, 20% or more of the ^{3}H label recovered in the liver extract was found to be incorporated into soluble nucleotides and RNA. Although in rat liver no cytidine deaminase can be detected [24], we found that the ^{3}H label entered the pools of uridine phosphates and of UDP-sugars to a considerable extent. [^{14}C]Orotic acid appeared to be a very good precursor for the uridine nucleotides, and radioactivity was also incorporated into cytidine nucleotides to a measurable extent. It was rapidly taken up by the liver and incorporated. Even after 5 min, not more than 10% of the radioactivity in the liver was unmetabolized orotic acid. On the basis of the body/liver weight ratio, the radioactivity from orotic acid accumulated in the liver, whereas that from uridine and cytidine did not. In Figs. 1(*b*) and 2(*a*)′, 2(*b*)′ and 2(*c*)′ the profiles of soluble radioactivity are shown after separation on Partisil-SAX and rechromatography on Lichrosorb respectively.

The specific radioactivities and the ^{14}C/^{3}H ratios of the various nucleotides were determined at several time

points after the injection of the radioactive precursors. The $^{14}C/^{3}H$ ratios for UDP and CDP could also be determined because either $NADP^+$, which is co-eluted with UDP, contained no label, or distinct peak fractions of CDP, well separated from UDP-glucuronic acid, could be used for the calculation. The ^{14}C specific radioactivities of UMP and UTP were high at 5 min and declined thereafter. The UDP-sugars contained at that time point a considerable amount of label, and the ^{14}C specific radioactivities increased with time. At 30 min the values for UTP and of the UDP-sugars were in the same range. The ^{14}C specific radioactivities of all soluble cytidine nucleotides were relatively low and did not vary much with time. In Table 1 the ^{14}C specific radioactivities are given for 5, 10 and 20 min. [^{3}H]Cytidine was readily incorporated into CMP and CTP and further into the cytidine compounds and RNA. The ^{3}H specific radioactivities of the soluble cytidine nucleotides were high from the beginning and remained of the same order for 30 min, and the differences between them were small. Therefore, the ^{3}H specific radioactivities and the accessory $^{14}C/^{3}H$ ratios only at 10 min are given in Table 1. Unexpectedly, incorporation of [^{3}H]cytidine varied much more from rat to rat than did that of [^{14}C]orotic acid.

DISCUSSION

Cytidine nucleotides are found in low concentrations in mammalian tissues, which hampers detection and reliable estimation of the concentration. In hepatocytes, cultured in monolayer the CDP-lipids even are below the detectable level (W. R. Pels Rijcken & W. Ferwerda, unpublished work). In addition, CMP-NeuAc is partly hydrolysed in cold PCA, a frequently used reagent for nucleotide extraction. Therefore studies concerning CXP-compounds and their metabolism are scarce. We developed an h.p.l.c. separation method which allowed us to separate and measure quantitatively most of the pyrimidine nucleotides, including all the CXP-compounds.

The concentrations of all cytidine nucleotides were in the range of 30–45 nmol/g wet weight, these concentrations being low in comparison with those of the uridine nucleotides. For CMP-NeuAc comparable values have been found with colorimetric quantification [13,25]. The values reported for CDP-choline, determined enzymically [26], and cytidine phosphates obtained with the h.p.l.c. technique [27,28] are in agreement with our results, although different rat strains were used. Values twice as high have been reported for CTP, determined enzymically, in the livers of female Wistar rats [26,29]. For the concentrations of the UDP-sugars we obtained higher values as most other investigators. Harms *et al.* [25] found the same concentration as we did for UDP-*N*-acetylhexosamine in livers of buffalo rats. In addition, our value found for UMP is also high. This might point to a high glycosylation rate in our rats, since UMP is the ultimate product of the nucleotide part in the lumen of the endoplasmic reticulum after glycosylation [30–32]. The concentration we found for UTP is similar to that found by others [27–29].

Using [^{14}C]orotic acid and [^{3}H]cytidine simultaneously as precursors of the synthesis *de novo* and the salvage pathway respectively, we tried to determine the labelling kinetics of the pyrimidine nucleotides and the involvement of the different metabolic flows. This elegant approach using the $^{14}C/^{3}H$ ratio as a measure of the involvement of the different flows, has been used by Losman & Harley [33] for hepatoma cells in culture. Although different cell types exist in liver tissue, the processes under investigation can mainly be attributed to the hepatocytes (W. R. Pels Rijcken & W. Ferwerda, unpublished work).

The ^{14}C specific radioactivities of the UDP-sugars became relatively high, taking into account that the label is substantial diluted in the large pools. This is indicative of a high turnover and/or an input of label from an UTP pool with a higher specific radioactivity than the measured average UTP value. A model with two UTP pools or flows given by Losman & Harley [33] might explain these results.

The kinetics of labelling with ^{14}C of the CTP pool is interesting. Although the average ^{14}C specific radioactivity of UTP remained much higher than of CTP, the value of the latter hardly increased, in spite of the fact that this small pool turns over rapidly because of the synthesis of RNA [19]. So, the ^{14}C label in [^{14}C]CTP from the *de novo* pathway is continually strongly diluted by CTP from the salvage pathway or CTP is synthesized from an UTP pool with a lower specific radioactivity than the average UTP value. It can be calculated that at 10 min only 2% of the incorporated ^{14}C radioactivity had been channelled from UTP to CTP. The ^{14}C specific radioactivity of CDP-choline at 5 min is somewhat higher than of CTP, whereas those of CDP-ethanolamine and CMP-NeuAc are lower. This might point to different flows of CTP, but the differences are small and the values soon converge.

For cytidine injected into rats a biological half-life has been determined of 25 h, whereas for uridine it is only 1 h [17]. When cytidine is taken up by the liver and incorporated into cytidine nucleotides, the radioactivity is relatively less diluted than that from orotic acid or uridine. These facts might explain the high ^{3}H specific radioactivities of the cytidine nucleotides at 5 min and the similar values at 10 and 20 min. We have no explanation for the great variations in ^{3}H incorporation we found from rat to rat. Probably this is due to biological variation. From the standard errors reported by Moyer *et al.* for rat [20] and mouse [34], similar variations in incorporation of uridine and cytidine can be concluded. Although the ^{3}H specific radioactivities of the uridine nucleotides are low, the percentage of the radioactivity channelled from [^{3}H]cytidine to the uridine nucleotides is much more (10%) than from UTP to the cytidine nucleotides (2%). In addition it can be calculated that a lot of the radioactivity, ^{14}C as well as ^{3}H, is incorporated into the nucleotide sugars and lipids. The total flow should even be greater, because some radioactive CMP and UMP is the product of glycosylation and lipidation processes. For a comparison of the different metabolic flows the $^{14}C/^{3}H$ ratio is a better parameter than the specific radioactivity. The ratio is independent of the inaccuracies introduced by the u.v.-absorption measurements and only partly influenced by the pool size and turnover rate. If a label remains for a relatively long time in a pool, because the pool is large and/or the turnover is low, a time-dependent effect has to be considered. In Table 1 the $^{14}C/^{3}H$ ratios are shown at 10 min. The ratio of UTP is lower than all ratios of the UDP-sugars and higher than that of 2′-+3′-UMP (RNA). The most logical explanation for liver tissue is intracellular com-

partmentation of the UTP pool, or, in other words, no complete mixing occurs of UTP derived from synthesis *de novo* and that from the salvage pathway. The flow of the salvage pathway is relatively more directed to RNA synthesis in the nucleus, and the flow of synthesis *de novo* relatively more to the cytoplasmic processes. Other investigators, mostly working with cells in culture, and using different approaches and different isotopic labelling, came to the same conclusion (for reviews, see [9] and [10]). The ratio of CTP also is higher than that of 2′- +3′-CMP (RNA), which is a strong indication that the CTP pool is compartmentalized too. The flow of CTP derived from the salvage pathway, metabolizing cytidine from the circulation, is directed to the nucleus for RNA synthesis to a relatively higher extent than the flow of CTP from synthesis *de novo*. The $^{14}C/^{3}H$ ratios of the CXP-compounds are difficult to interpret. The ratio of CMP-NeuAc is lower than that of CTP and approximates to the low value of 2′-+3′-CMP (RNA). This might point to a nuclear-localized synthesis of CMP-NeuAc. However, the low value of CDP-ethanolamine, supposed to be synthesized in an extranuclear locale, hampers an obvious conclusion. Further study is needed to clarify the situation.

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