ENZYMES OF URACIL METABOLISM IN TISSUES WITH DIFFERENT GROWTH CHARACTERISTICS

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SUMMARY

Different tissues were investigated for their contents of uridine phosphorylase, uridine kinase, deoxyuridine phosphorylase and deoxyuridine kinase activities. These enzyme contents were considered to mirror the relative efficiency of an enzymic pathway using uracil for the biosynthesis of nucleic acid pyrimidines. Furthermore, the same tissues were investigated for their capacities to convert orotic acid to UMP, representing the *de novo* pathway of pyrimidine synthesis. In a survey of some normal tissues from rat, mouse and rabbit, a certain degree of correlation was found between the efficiency of this alternative pathway and the growth rate.

During liver regeneration, marked increases in the enzymes studied were observed. Thus, uridine phosphorylase showed a maximal threefold increase between 36 and 72 h after hepatectomy. Corresponding values were a fivefold increase for uridine kinase (24–36 h), a fourfold increase for deoxyuridine phosphorylase (about 48h), a tenfold increase for deoxyuridine kinase (36–48 h) and, for the enzymes transforming orotic acid to UMP a 5- to 6-fold increase (about 48 h). 144 h after the operation, all enzymes had reverted to almost normal values. These rapid changes in the activities of enzymes involved in the synthesis of nucleotides, might form part of an homeostatic mechanism involved in pyrimidine biosynthesis.

INTRODUCTION

The function of free uracil as an intermediate in polynucleotide synthesis has been the subject of many investigations. Originally PLENTL AND SCHOENHEIMER[1] were unable to find any incorporation of [^{15}N]uracil into rat polynucleotide pyrimidines *in vivo*. Later studies in different laboratories[2–5] with rat tissues *in vivo* and *in vitro* showed a small but definite incorporation of [2-^{14}C]uracil which in most cases, was much smaller than that of orotic acid or pyrimidine nucleosides. With high uracil concentrations, however, CANELLAKIS[6] demonstrated that rat-liver slices utilized this pyrimidine to about the same extent as orotic acid. Even at moderate dose levels, a high incorporation of uracil was observed *in vivo* in rat intestinal mucosa, Flexner-Jobling carci-

Abbreviations: UMP and dUMP, uridine 5′-monophosphate and deoxyuridine 5′-monophosphate respectively; ADP and ATP, adenosine 5′-di- and -triphosphate respectively; PGA, 3-phosphoglyceric acid; PCA, perchloric acid; Tris, tris(hydroxymethyl)aminomethane; RNA ribosenucleic acid; DNA, deoxyribosenucleic acid; P_i, inorganic phosphate.

noma[7], mouse tissues and, most pronounced, in the Ehrlich ascites tumour[8]. The reactions leading to the synthesis of UMP can be regarded as the first steps in the incorporation of free uracil into polynucleotides[9,10].

In earlier work from this laboratory the following two reactions were shown to be catalyzed by extracts of Ehrlich ascites tumour[11]:

$$\text{Uracil} + \text{ribose-1-phosphate} \xrightarrow[\text{phosphorylase}]{\text{uridine}} \text{uridine} + \text{phosphate}$$

$$\text{Uridine} + \text{ATP} \xrightarrow[\text{kinase}]{\text{uridine}} \text{UMP} + \text{ADP}$$

In spite of the fact that both uridine phosphorylase[12–14] and uridine kinase[12,15] are known to occur in extracts of mammalian liver we could only find a small conversion of uracil to UMP with extracts from rat and mouse liver. From our results[16] it also appeared that in rat liver, uridine kinase activity represented a rate limiting step for UMP synthesis. It could be demonstrated that considerably higher levels of both uridine phosphorylase and kinase occurred in ascites tumour extracts than in rat-liver extracts. This was most pronounced for uridine kinase which, on a protein basis consistently showed 5–30 times higher activity in tumour extract. It thus became clear that the enzyme pattern in the ascites tumour favoured the utilization of uracil for polynucleotide synthesis and it was suggested that this might reflect an adaptation of the tumour to the high requirements of nucleic acid synthesis.

The aim of the present investigation is to assess the relative efficiency of uracil utilization as an alternative pathway for polynucleotide synthesis. For this purpose, the levels of the enzymes transforming uracil to UMP and deoxy-UMP were determined in extracts from different tissues. For comparison, a similar study was made of enzymes transforming orotic acid to UMP. The tissues studied were normal tissues from mouse, rat and rabbit as well as rat liver at different stages of regeneration after partial hepatectomy.

EXPERIMENTAL PROCEDURE

[2-^{14}C]uracil and non-labelled uridine and deoxyuridine were purchased from Schwarz Laboratories, Inc.

[2-^{14}C]deoxyuridine was synthesized enzymically from [2-^{14}C]uracil and non-labelled deoxyuridine by an exchange reaction catalyzed by extracts from *E. coli*[4]. A typical procedure ran as follows: [2-^{14}C]uracil (260 μmoles) and deoxyuridine (260 μmoles) were dissolved in 120 ml of 0.05 *M* Tris buffer pH 7.4. 120 μmoles of phosphate buffer pH 7.4 and 35 ml of *E. coli* extract* corresponding to a total of 1225 mg of wet bacteria were added. Incubation was carried out for 7 h at 37°. Uracil and deoxyuridine were separated by chromatography on starch. After stopping the reaction by heating on a boiling water bath, the incubation mixture was divided in four portions which were chromatographed separately on starch columns (4 cm × 30 cm). Elution was carried out with ammonium acetate–ethanol, pH 9.5. (32 ml of

* *E. coli* K 12 were obtained through the courtesy of Dr T. HOLME, Department of Bacteriology, Karolinska Institutet, Stockholm (Sweden). The bacteria were grown on a synthetic medium and harvested by centrifugation. The cells were disrupted in the press described by EDEBO[17] and finally extracted at 0° with 0.0062 *M* phosphate buffer at pH 7.1. Cell debris was removed by centrifugation at 20,000 × *g* for 15 min at 0°.

5 *M* ammonium acetate, pH 9.5, 352 ml of 95 % ethanol and 112 ml of water). The deoxynucleoside emerged from the column with an R-value of about 1.8 immediately followed by the pyrimidine. After rechromatography in the same system, deoxyuridine essentially free from uracil, was obtained. A complete equilibration of isotope between uracil and deoxyuridine occurred.

[2-^{14}C]uridine was synthesized enzymically by an exchange reaction analogous to the one described above. [2-^{14}C]uracil (90 μmoles) and uridine (100 μmoles) were dissolved in 60 ml of 0.05 *M* Tris buffer pH 7.4. 60 μmoles of phosphate buffer pH 7.4 and 14 ml of *E. coli* extract corresponding to 490 mg of wet bacteria were added. Incubation for 3 h at 37°. The reaction was stopped by heating on a boiling water bath for 3 min. Separation of uracil and uridine was performed by ion exchange chromatography. The reaction mixture was introduced to a Dowex-2-chloride (200–400 mesh) column 2.5 cm in diameter and 12 cm of length. The column was previously equilibrated with 0.04 *M* ammonium hydroxide–0.01 *M* ammonium chloride–0.001 *M* sodium tetra borate pH 10.2. The pyrimidine was eluted after circa 14 column volumes of 0.2 *M* ammonium hydroxide–0.05 *M* ammonium chloride–0.001 *M* sodium tetra borate, pH 10.0. The column was then washed thoroughly with water and the nucleoside finally eluted with 0.05 *M* acetic acid. Equilibration of radioactivity between uracil and uridine was obtained during the incubation. The recovery of radioactivity was usually about 90 %.

Ribose-1-phosphate was prepared enzymically as the barium salt with nucleoside phosphorylase from calf spleen[18].

[2-^{14}C]orotic acid was a preparation available in the laboratory. It was originally synthesized according to NYC AND MITCHELL[16].

Preparation of extracts

Most of the experiments were carried out with extracts of acetone powders from the tissues studied. The preparation of these powders and their extraction was described earlier[11,16]. The amount of proteins extracted during this procedure was checked by Kjeldahl analysis.

In the experiments on regenerating rat liver "supernatant fractions" of tissue homogenates were used as described previously[16]. Partial hepatectomy was performed on male albino rats (150–200 g) by removing of the medial and left lateral liver lobes and the posterior lobule of the right lateral lobe.

The Ehrlich ascites tumour was obtained through the courtesy of Prof. G. KLEIN, Department of Tumour Biology, Karolinska Institutet, Stockholm (Sweden).

Determination of enzyme activities

Uridine phosphorylase and uridine kinase: These two enzyme activities were estimated simultaneously by paper chromatographic separation of the radioactive products after enzymic phosphorolysis and phosphorylation of [2-^{14}C]uridine. For this purpose 10.5 μmoles of $MgCl_2$, 2.1 μmoles of ATP, 6.4 μmoles of PGA, 4.3 μmoles of phosphate buffer pH 7.4, 7.5 μmoles of Tris buffer pH 7.4 and [2-^{14}C]uridine (usually 0.45 μmoles) were incubated with tissue extract at 37° for 15 min in a final volume of 0.26 ml. The reaction was stopped by the addition of 0.03 ml of 4 *M* PCA. The supernatant was centrifugated and heated at 100° for 1 h to break pyrophosphate and purine glycosyl bonds. After cooling on ice, the supernatant was neutralized

(indicator paper) with 4 *M* KOH, and $KClO_4$ allowed to precipitate. Aliquots (0.03 ml) and nonlabelled carriers were subjected to descending paper chromatography on Whatman No. 1 filter paper[16]. A complete separation of uracil, uridine and UMP occurred. The different spots were detected by a Mineralight u.v.-lamp, their corresponding areas cut out and eluted with water. Several aliquots from these eluates were plated and counted at infinite thinness in a Tracerlab Sc-18 windowless flow counter. The relative amounts of labelled uracil, UMP and uridine could thus be determined in each run. By applying these values to the original amount of substrate, the amounts of uracil and UMP produced were calculated.

Deoxyuridine phosphorylase and deoxyuridine kinase: The general procedure for the determination of these enzymes was identical with the one described above. However, [2-^{14}C]deoxyuridine was used as substrate and the deproteinized supernatants were not subjected to hydrolysis at 100°. The acid hydrolysis could be omitted because dUMP-pyrophosphates are not formed enzymically[20]. Since the solvent used gave a borderline separation of uracil and deoxyuridine, a blank sample without enzyme was always run parallel and the uracil activity of the blank was subtracted from the sample uracil value*. Due to the low activities of the deoxyuridine kinase the incubation period in most experiments was prolonged to 30 min.

Transformation of orotic acid to uridine phosphates: The formation of labelled uridine phosphates from [2-^{14}C]orotic acid was measured mainly according to HURLBERT AND REICHARD[15]. Thus 3.8 μmoles of ribose-5-phosphate, 3.8 μmoles of ATP, 11.4 μmoles of PGA, 9.5 μmoles of $MgCl_2$, 10.0 μmoles of Tris buffer pH 7.4 [2-^{14}C]orotic acid (usually 0.16 μmoles) and enzyme were incubated for 30 min at 37° in a final volume of 0.42 ml. After stopping the reaction with 0.05 ml of 4 *M* PCA the supernatant was treated after centrifugation in the same way as described above for the uridine enzyme procedure. Orotic acid, UMP and uracil + uridine (formed through dephosphorylation of UMP) were separated on paper with the ammonium acetate–ethanol solvent described above for column chromatography separation of uracil and deoxyuridine. (R_F-values: orotic acid approx. 0.40; UMP approx. 0.14 and uracil + uridine approx. 0.65). The amount of labelled uracil + uridine was always found to be negligible compared to the amount of [2-^{14}C]UMP.

Catabolism of uracil: The enzymic degradation of [2-^{14}C]uracil was measured as described previously[16]. Thus $^{14}CO_2$ from [2-^{14}C]uracil was collected in 4 *M* KOH in the centre well of a Warburg vessel, precipitated as $BaCO_3$ and counted at infinite thickness in an end-window Geiger counter.

RESULTS

Validity of assay methods

pH-optimum of uridine phosphorylase: The pH-optimum of the reaction:

$$\text{Uridine} + P_i \rightleftarrows \text{uracil} + \text{ribose-1-phosphate}$$

was measured in both directions (Fig. 1) using acetone powder extracts from ascites tumour as the enzyme source. For details of experimental procedure see explanation of Fig. 1. The reaction in both directions has a rather broad pH-optimum about pH 8.

* The blank showed values of 2–3 % of total radioactivity as uracil and less than 0.2 % as dUMP.

A pH-value of 7.4 which was used in the determinations of uridine phosphorylase activities lies quite close to the optimum.

Dependence of uridine phosphorylase and deoxyuridine phosphorylase on phosphate concentration: In Fig. 2 the enzymic phosphorolysis of uridine and deoxyuridine were measured as a function of phosphate concentration. A dialyzed extract from acetone

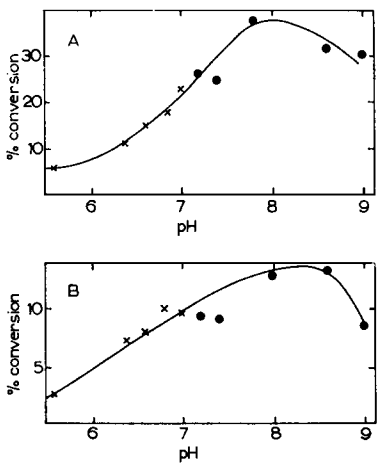

Fig. 1. pH curve of uridine phosphorylase. A. Formation of uridine from uracil and ribose-1-phosphate. Substrate: [2-^{14}C]uracil 1.5 μmole, ribose-1-phosphate 0.8 μmole, buffer (x, imidazole; o, Tris) 50 μmoles. Incubation at 37° 15 min, with 3.0 mg enzyme (acetone powder from ascites tumour). Volume 0.28 ml. B. Phosphorolysis of uridine. Substrate: [2-^{14}C]-uridine, 0.42 μmole, phosphate 10.0 μmoles, buffer 100 μmoles (x, imidazole; o, Tris). Incubation at 37° for 15 min with 3.0 mg enzyme (acetone powder from ascites tumour). Volume 0.23 ml.

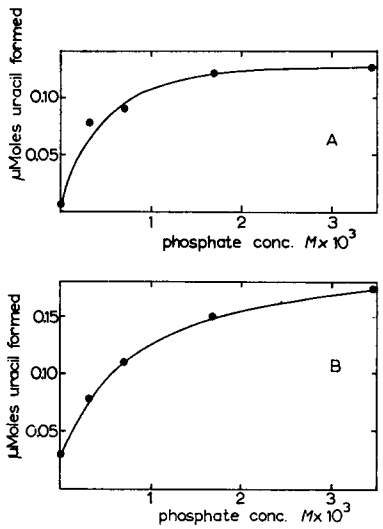

Fig. 2. A. Dependence of uridine phosphorylase on phosphate. Substrate: [2-^{14}C]uridine 0.45 μmole. Tris buffer 10.0 μmoles, pH 7.4, and phosphate as indicated on abscissa. Incubation for 15 min at 37° with 2.2 mg enzyme (dialyzed extract from acetone powder from ascites tumour). Volume 0.26 ml. B. Dependence of deoxyuridine phosphorylase on phosphate. Substrate: [2-^{14}C]deoxyuridine 0.47 μmole. Tris buffer 10.0 μmoles, pH 7.4, and phosphate as indicated on abscissa. Incubation for 30 min at 37° with 7.4 mg enzyme (dialyzed extract from acetone powder from ascites tumour). Volume 0.26 ml.

powder of ascites tumour (3 ml of extract dialyzed for 24 h against two 2-l changes of 0.05 *M* Tris buffer pH 7.4) was used as an enzyme source. Both enzyme activities approach a plateau which was reached at a phosphate concentration of about 0.002 *M*. In the subsequent determinations of the two phosphorylases, a phosphate concentration of 0.017 *M* was used.

Enzymic formation of uracil and UMP from uridine: In all determinations of uridine phosphorylase and uridine kinase a wide range of enzyme concentrations was used to ensure the rectilinear portion of the enzyme concentration–activity curve. Fig. 3 shows the formation of uracil and UMP with different amounts of enzyme in two typical experiments. The relative amounts of labelled UMP, uracil and uridine are plotted against the amount of extract used. From the initial slopes of the curves the two enzyme activities were expressed as amounts of uracil or UMP formed per mg of acetone powder during a period of 15 min. In control experiments without

enzyme there was no [2-^{14}C]UMP activity although a small amount of [2-^{14}C]uracil was sometimes found which approx. corresponded to 0.2 % of the total radioactivity. The amount of [2-^{14}C]uridine introduced initially was always carefully standardized by u.v. spectrophotometry and gave an initial concentration well above that required for saturation as stated earlier[16].

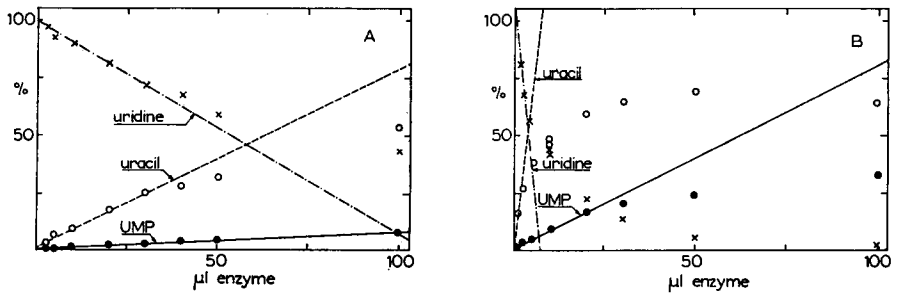

Fig. 3. Enzymic formation of uracil and UMP from uridine at different enzyme concentrations. Relative amounts of uridine, uracil and UMP (ordinate) isolated after incubation with different amounts of enzyme (abscissa). For details of procedure see the text under EXPERIMENTAL. Enzyme: acetone powder extract from rabbit bone marrow 125 mg/ml (A) and from mouse intestine 125 mg/ml (B).

Identical conditions were shown to be valid for the formation of uracil and dUMP from deoxyuridine.

ATP level during uridine kinase assay: Failure of the ATP-regenerating system in the assay mixture would give erroneous low values for uridine kinase activity. In order to check this point, 0.1 ml of the "supernatant fraction" from normal rat liver was incubated with the usual assay system without uridine. The usual procedure was followed but the acid hydrolysis at 100° omitted. After neutralization, the supernatant was chromatographed on a Dowex-2-formate column according to HERBERT *et al.*[22]. In this experiment the presence of 0.1 µmoles of ADP and 1.5 µmoles of ATP could be demonstrated after incubation (2.1 µmoles of ATP were originally added).

Survey of normal tissues from mouse, rat and rabbit

All experiments in this section were carried out with acetone powder extracts from the different tissues.

Uridine phosphorylase and uridine kinase: The results for these two enzymes are summarized in Table I. It is evident that heart muscle, brain and liver contained relatively low levels of both enzymes. The other extreme position was held by mouse intestine and Ehrlich ascites tumours which showed uridine kinase activities 5–15 times greater than those of mouse liver. Mouse intestine gave by far the highest uridine phosphorylase value*. Kidney and spleen occupy an intermediate position with respect to both enzymes and finally bone marrow from rat and rabbit showed values comparable to those of rat liver. These low values were surprising in view of the high rate of cell turnover known to take place in bone marrow and which might

* This activity represents a true phosphorolysis of uridine and is not caused by contamination with *e.g.* bacterial hydrolases[21]. Thus, after extensive dialysis the formation of uracil was stimulated more than tenfold by the addition of inorganic phosphate.

indicate that bone marrow depends heavily on *de novo* synthesis of pyrimidines. On the other hand, the preparation of acetone powder from bone marrow was difficult and time consuming and the observed values might be too low.

TABLE I

DETERMINATIONS OF URIDINE KINASE AND URIDINE PHOSPHORYLASE IN ACETONE POWDER EXTRACTS FROM TISSUES OF MOUSE, RAT AND RABBIT

Activities as mμmoles of uracil or UMP formed per mg acetone powder/15 min.

	Uridine kinase	*Uridine phosphorylase*	*mg N/ml/100 mg**
Bone marrow (rabbit)	2.7	26.9	3.60
Bone marrow (rat)	8.0	5.4	—
Heart muscle (mouse)	3.8	10.3	5.44
Brain (mouse)	9.5	3.0	4.85
Liver (rat)	2.5	14.2	9.33
Liver (mouse)	9.3	12.8	8.25
Kidney (mouse)	12.4	17.1	7.70
Spleen (mouse)	31.0	5.9	7.50
Intestine (mouse)	51.0	346.0	7.26
Ehrlich ascites tumours	50–140	50–140	—

* The extracts were made from different amounts of acetone powder. In order to make the nitrogen analyses directly comparable these values are corrected to an assumed acetone powder content of 100 mg/ml.

TABLE II

DEOXYURIDINE PHOSPHORYLASE, DEOXYURIDINE KINASE AND FORMATION OF UMP FROM OROTIC ACID IN TISSUES OF MOUSE, RAT AND RABBIT

Activities as mμmoles of uracil, dUMP and UMP respectively formed per mg of acetone powder per 15 min.

	Deoxyuridine kinase	*Deoxyuridine phosphorylase*	*UMP from orotic acid*
Bone marrow (rabbit)	0.1	1.3	0.2
Heart muscle (mouse)	0.1	1.2	0.2
Liver (rat)	0.1	5.3	0.2
Kidney (mouse)	0.2	2.1	0.1
Spleen (mouse)	0.3	0.5	1.7
Intestine (mouse)	0.4	90.4	0.2
Ehrlich ascites tumours	1–2	10–30	1–5

Deoxyuridine phosphorylase and deoxyuridine kinase

Table II gives a summary of determinations of these two enzymes carried out with extracts from different tissues. The same general pattern as for uridine phosphorylase and uridine kinase can be recognized. Thus, mouse intestine and Ehrlich ascites tumours gave the highest values for both deoxyuridine phosphorylase and deoxyuridine kinase. Relatively low levels in bone marrow were observed with these enzymes also.

Formation of UMP from orotic acid: These determinations were made as a relative measure of the capacity of the different tissues to utilize orotic acid for nucleic acid synthesis. The transformation of orotic acid to UMP involves only a small part of the *de novo* pathway for the synthesis of pyrimidines. Furthermore, a sum of three enzyme reactions[23,24] is measured simultaneously. If low conversion

rates were ascertained, however, the results were reproducible and proportional to enzyme concentrations. The last column of Table II gives determinations of the enzymes in different tissues. It can be seen that a rough parallel existed between the amounts of these enzymes and the phosphorylases and kinases in bone marrow, heart muscle, liver and kidney. Spleen, however, gave a very high activity while intestine showed a rather low value. These results would indicate a preponderance of *de novo* synthesis of pyrimidines *via* orotic acid in spleen, while in the intestine most polynucleotide pyrimidines could be synthesized from preformed uracil, arising *e.g.* from the diet.

Studies on regenerating liver

After hepatectomy the remaining lobe was allowed to regenerate for different periods of time before the animals were killed. These periods were usually multiples of 12 h in order to obtain the enzyme analyses in phase with the diurnal variations of the mitotic index in regenerating rat liver[25]. At each time point chosen, three rats were sacrificed, the liver lobes removed and immediately cooled on ice. Acetone powders were made from one part of the pooled lobes within each group. The pooled rests were homogenized and the "supernatant fractions" were prepared. These supernatants usually showed a nitrogen content of 2.5–3.0 mg N/ml.

Uridine phosphorylase and uridine kinase: Fig. 4 shows uridine phosphorylase and uridine kinase activities in the "supernatant fractions" from regenerating rat liver at different intervals after partial hepatectomy. Both enzymes were measured simultaneously and the two curves of Fig. 4 thus represent the same series of operated animals. The activities are expressed as μmoles of uracil and UMP respectively formed per mg of protein-N during a 15-min period. A sharp rise in uridine phosphorylase occurs after 24 h after the operation with maximal activities between 36 and 72 h. After that, enzyme activity proceeded to decrease slowly and at the 144-h time

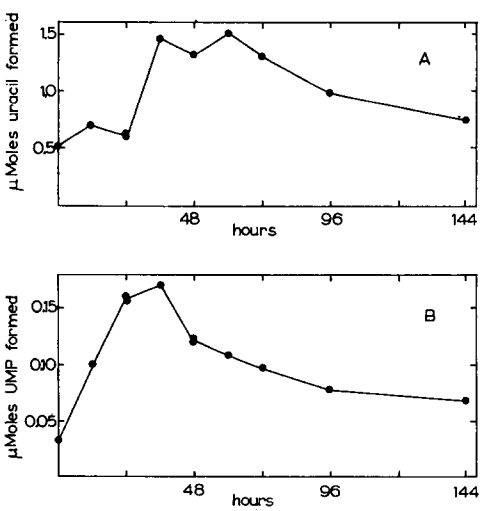

Fig. 4. Enzymic formation of uracil (A) and UMP (B) from uridine with extracts from regenerating liver. Ordinate: μmoles of uracil and UMP, respectively, formed per mg of protein nitrogen. Abscissa: regeneration period in hours. Enzyme: "supernatant fractions" from regenerating rat liver containing 2.3–2.8 mg protein-N/ml. For details of procedure see the text under EXPERIMENTAL.

point the activity was almost back to normal. Fig. 4B gives the corresponding curve for uridine kinase. The peak activity of the enzyme was reached earlier than that of phosphorylase. Thus, a rise had already begun 12 h after surgery. Maximal activities were measured at 24 and 36 h,followed by a steady decline. It should be noted that, at every time point uridine phosphorylase exhibited activities which were about ten times higher than those of uridine kinase.

Deoxyuridine phosphorylase and deoxyuridine kinase: These enzymes were measured in extracts of acetone powders from regenerating rat liver. This allowed experiments with much more concentrated protein solutions than was possible with "supernatant fractions". The procedure was adopted because of the small quantities of these activities present in liver. Fig. 5A shows deoxyuridine phosphorylase activities. At 48 h after surgery, a rise, about four times greater than the activity curve of nonregenerating liver can be seen. At 144 h, the activity had almost reverted to that of normal liver. A similar curve was obtained for deoxyuridine kinase (Fig. 5B). This enzyme showed the most marked rise in activity of all the enzymes studied. The maximum was reached after 36 h and represented a tenfold rise over normal liver. Deoxyuridine phosphorylase activity was 10–20 times greater than that of deoxyuridine kinase throughout the whole regeneration period.

Formation of UMP from orotic acid: These determinations were also performed with acetone powder extracts. Fig. 6 shows a sharp peak in enzyme activity at 48 h after hepatectomy. The activity then drops sharply and has reached almost normal values 70–80 h after the operation.

Catabolism of uracil: From the "supernatant fractions" used for the analyses of uridine phosphorylase and uridine kinase, samples were withdrawn and immediately tested for their ability to decompose uracil to CO_2 as described earlier[16]. As can be seen from Fig. 7, the capacity of liver to decompose uracil began declining after 24 h.

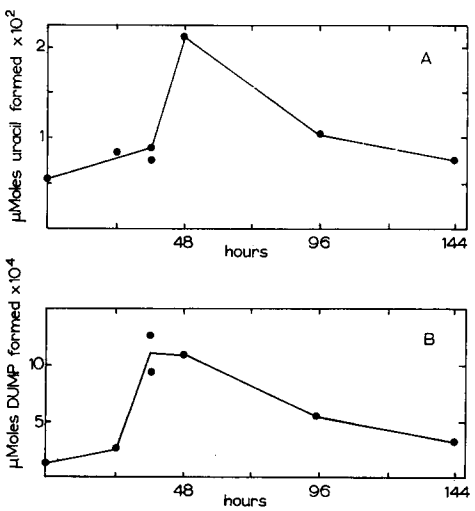

Fig. 5. Enzymic formation of uracil (A) and dUMP (B) from deoxyuridine with extracts from regenerating liver. Ordinate: µmoles of uracil and dUMP, respectively, formed per mg of acetone powder. Abscissa: regeneration period in hours. Enzyme: acetone powder extracts from regenerating liver 166 mg/ml. For details of procedure see the text under EXPERIMENTAL.

A minimum is reached after 48–60 h, after which time a slow rise takes place. Normal values were again obtained after 144 h. This curve is a confirmation of the earlier results of CANELLAKIS[26].

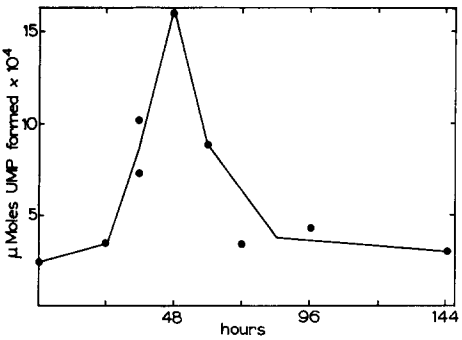

Fig. 6. Enzymic formation of UMP from orotic acid. Ordinate: μmoles of UMP formed/mg acetone powder. Abscissa: regeneration period in hours. Enzyme: acetone powder extracts from regenerating liver, 166 mg/ml.

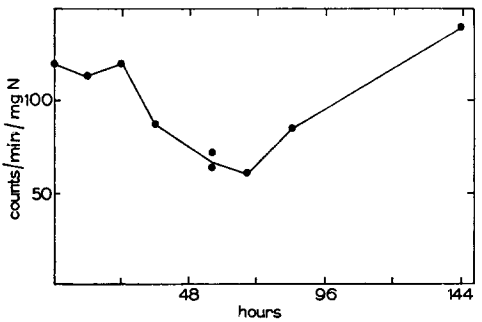

Fig. 7. Enzymic formation of $^{14}CO_2$ from [2-^{14}C]uracil with extracts from regenerating liver. Conditions as described under EXPERIMENTAL. Ordinate: counts/min ($BaCO_3$ at infinite thickness) per mg protein nitrogen in extract. Abscissa: regeneration period in hours.

Each point is calculated from three experiments performed with 0.3 ml 0.5 ml and 1.0 ml respectively of "supernatant fraction" from regenerating liver (2.3–2.8 mg protein nitrogen/ml).

DISCUSSION

The present investigation was undertaken with the primary aim of investigating whether tissues with different growth characteristics showed variations in their ability to utilize uracil for nucleotide synthesis. Such an alternative pathway, which utilizes the combined action of nucleoside phosphorylases and kinases, might possibly be regarded as a "reserve mechanism" for nucleic acid synthesis in tissues with rapid growth rates. As discussed earlier[16], uracil is probably not formed *de novo* from small molecules.

The values of Table I for uridine phosphorylase and uridine kinase activities in different tissues show a correlation in some cases between these enzymes and the growth rate. Consistent with earlier results[16], the rapidly growing ascites tumours gave the highest relative values. The high values obtained for intestine are also compatible with its high growth rate (mucosal cell renewal in 35 h[27]) and the good incorporation of free uracil observed earlier[2,7]. However, bone marrow with its very rapid cell turnover showed quite low enzyme values. Tissues with low growth rates, such as heart muscle, brain and liver, showed low enzyme activities.

The corresponding analyses for deoxyuridine phosphorylase and deoxyuridine kinase shown in Table II demonstrate the same general pattern. Thus, ascites tumours and intestine showed the highest relative values of both enzymes. In this case, too, bone marrow gave low values, contrasting with its rapid cell turnover.

The capacities of the different tissues to convert orotic acid to UMP were investigated as representative for the relative efficiency of the *de novo* synthesis of nucleic acid pyrimidines. If these conversion capacities (Table II) are compared to the general pattern of phosphorylase and kinase activities, two striking differences emerge. Intestine gives a very low value and spleen a relatively high one, compared to the

relative concentrations of phosphorylases and kinases in these tissues. These data might reflect a strong domination of the uracil utilizing pathway in intestine and of the orotic acid pathway in spleen.

Regenerating liver has a very rapid growth rate, it is capable of doubling its weight in about 48 h[28]. In correlation with this fact, the enzymes leading to the synthesis of UMP and dUMP showed greatly elevated tissue concentrations during liver regeneration. All the enzymes studied reverted to levels close to those of normal liver when the regeneration was complete. Uridine kinase showed an earlier and more distinct peak than uridine phosphorylase. The maximum was reached between 24 and 36 h after surgery compared to between 36 and 72 h for uridine phosphorylase. Moreover the total increase is 5.3 fold as compared to 3 fold for uridine phosphorylase. The maximum for the kinase almost coincides with those for RNA-pyrimidine turnover[29] and amino acid incorporation[30] observed earlier in regenerating liver, which both occurred 30 h after operation. However, the main increase in uridine phosphorylase activity occurred definitely after those events. Thus, after 24 h, no certain increase had occurred, even though at that time RNA-pyrimidine synthesis is far above normal, as measured by the incorporation of different precursors. A picture similar to that of uridine phosphorylase was obtained for the enzymes involved in the transformation of orotic acid to UMP and for deoxyuridine phosphorylase and kinase. All these enzymes showed rather late maxima. The maxima for the latter two enzymes should be compared with the maximum of DNA-synthesis, which occurs at 24 h[29]. This time lag, however, is in agreement with the observations of BOLLUM AND POTTER[31] that thymidine incorporating enzymes in regenerating liver continued to increase while DNA-synthesis *in vivo* was decreasing. The total increase in thymidine incorporating enzymes was by factors of 10–25 or more over normal nonregenerating liver. The observed tenfold increase in deoxyuridine kinase in the present experiments is consistent with the pronounced increase in thymidine kinase observed by CANELLAKIS[26]. It seems quite likely that the phosphorylation of thymidine and deoxyuridine is carried out by the same enzyme.

It should be pointed out that both uridine and deoxyuridine kinases in regenerating liver in general have an activity 10–20 times lower than the corresponding phosphorylases. This, together with their earlier increase during regeneration, might reflect their rate-limiting character in the utilization of uracil for the synthesis of nucleic acid pyrimidines.

The determinations of uracil catabolism during liver regeneration reproduce rather closely the results obtained earlier by CANELLAKIS *et al.*[26]. Thus, a 50% decrease was found between 48–60 h after hepatectomy. CANELLAKIS[6,26] has suggested that the increased incorporation of uracil into RNA during liver regeneration might be due to the diminished catabolism of uracil. In the present investigation, evidence is given for greatly increased tissue concentrations of the enzymes involved in the anabolism of uracil during liver regeneration. It is probable that the homeostatic mechanism involved in pyrimidine synthesis is dependent on both the decrease of uracil catabolism and the increase in the capacity to anabolize uracil.

All determinations in this work were carried out with cell extracts. The information was obtained from experiments which aimed at the elucidation of enzyme activities at optimal conditions of substrate concentrations. These conditions do not necessarily prevail to the same extent in all cells studied. Such considerations make it

difficult to use the information obtained in the present experiments for conclusions concerning the relative efficiencies of the orotic acid pathway and uracil pathway *in vivo* in different tissues. It is nevertheless considered that approximative comparisons between different cells can be made as far as the same enzyme, or possibly enzyme sequence is concerned. These considerations of relative efficiencies of metabolic routes for the synthesis of nucleic acid pyrimidines in different tissues might be of some import to the discussion on the mechanisms of growth inhibition by pyrimidine analogues.

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