ENZYMES OF URACIL METABOLISM IN THE EHRLICH ASCITES TUMOUR AND MAMMALIAN LIVER*

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The ability of various tissues to utilize uracil for polynucleotide synthesis has been studied on several occasions. Originally PLENTL AND SCHOENHEIMER[1] demonstrated that ^{15}N-labelled uracil when injected into rats did not specifically contribute any of its nitrogen to polynucleotide pyrimidines. Later investigators, using ^{14}C-labelled uracil[2–5], found a certain amount of incorporation into rat tissues both *in vivo* and *in vitro*. In most cases the incorporation was considerably smaller than that of pyrimidine nucleosides and orotic acid (see *e.g.* ref. [4]). Using large amounts of uracil however, CANELLAKIS[6] has recently demonstrated that rat liver slices used this pyrimidine as well as orotic acid for polynucleotide synthesis. A much better utilization of uracil, even at moderate dose levels, has been demonstrated with mouse tissues and especially with the Ehrlich ascites tumour[7].

In a previous investigation we have studied the enzymic formation of uridine monophosphate (UMP)** from uracil in extracts of the Ehrlich ascites tumour. The work of HURLBERT AND POTTER[8] and OCHOA[9] indicates that uridine phosphates are closely involved in the synthesis of polynucleotides; UMP formation was therefore considered to be the first step in the transformation of free uracil to polynucleotide pyrimidines. We could demonstrate the following two reactions in the tumour extracts[10]:

$$\text{Uracil} + \text{ribose-1-phosphate} \xrightarrow[\text{phosphorylase}]{\text{pyrimidine nucleoside}} \text{Uridine} + \text{phosphate} \qquad (1)$$

$$\text{Uridine} + \text{ATP} \xrightarrow[\text{kinase}]{\text{uridine}} \text{UMP} + (\text{ADP}) \qquad (2)$$

Under our experimental conditions only very small amounts of UMP were formed from uracil in extracts from rat liver or mouse liver, even though it is known that both nucleoside phosphorylase[11,12] und uridine kinase[6,13] are present in extracts from mammalian liver. In the present investigation we have tried to clarify the reasons for these variations in uracil utilization by determining the amounts of the two enzymes in the different tissues. A comparison was also made of the capacity of the tissues to degrade uracil, since CANELLAKIS[6] has suggested that the catabolism of uracil might be partly responsible for its low incorporation into polynucleotides by rat liver.

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** The following abbreviations are used in this paper: UMP, UDP and UTP for uridine-5-mono-, di- and triphosphate, respectively. AMP, ADP and ATP for adenosine-5′-mono-, di- and triphosphate, respectively. CMP, cytidine-5′-monophosphate; PGA, 3-phosphoglyceric acid; PCA, perchloric acid; Tris, tris(hydroxymethyl)aminomethane; TPN, triphosphopyridine nucleotide; TPNH, reduced TPN; RNA, ribosenucleic acid.

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EXPERIMENTAL

Materials

Uracil-2-^{14}C was synthesized according to JOHNSON AND FLINT[14]. Uridine-2-^{14}C was prepared from orotic acid-2-^{14}C via UMP as described by HURLBERT AND REICHARD[13]. UMP was dephosphorylated with snake venom phosphatase (*Crotalus adamanteus*). The uridine obtained in this way was purified by starch chromatography[15].

Nonlabelled uridine was a gift from Dr. U. LAGERKVIST and had been prepared according to BREDERECK[16]. TPN and ATP were commercial samples (Sigma Chemical Company).

Preparation of extracts

The Ehrlich ascites tumour cells were obtained through the courtesy of Dr. A. FORSSBERG, Institute of Radiophysics, Karolinska Institutet, Stockholm, Sweden. The preparation of the acetone-dried powders from the ascites tumour cells and liver has been described previously[10]. The acetone powders were stored in a desiccator at 0°. They were extracted within a few days after preparation, since it was found that at least some of the enzymes studied deteriorated quite rapidly. Extraction was usually carried out in a Potter-Elvehjem homogenizer with 10 vol. of 0.05 *M* tris buffer, pH 7.4, in an ice bath. After 30 min the solution was centrifuged at 20,000 *g* for 10 min at 0° and the clear extract was then immediately used for the experiments. A relative measure of the amount of protein in the extracts was obtained by nitrogen analysis. In the case of the determination of nucleoside phosphorylase activity the extract was dialysed for 24 hours at +5° against a total of 4 l of glass-distilled water.

Some experiments were also carried out with the "supernatant fractions" of tissue homogenates without the preparation of acetone powders. For this purpose the livers were homogenized in 4 vol. of 0.05 *M* tris buffer, pH 7.4, containing 0.11 *M* KCl. The ascites tumour was homogenized by treatment in a Raytheon 10-kc magnetostriction oscillator for 7–8 min. The supernatant obtained after centrifugation at 100,000 *g* (30 min) in a Spinco centrifuge was directly used for the experiments.

Determination of enzyme activities

Nucleoside phosphorylase. The spectrophotometric method devised by FRIEDKIN AND ROBERTS[17] for thymidine phosphorylase was adapted to our purpose. In this method the light absorption at 300 mμ at pH 13–14 is measured. When corrected by means of an appropriate blank the absorption at this wavelength is directly proportional to the formation of uracil from uridine.

Uridine (50 μmoles) and 100 μmoles of phosphate buffer, pH 7.4, were incubated together with the dialysed enzyme in a final volume of 2.0 ml at 37°. At different time intervals 0.3 ml of the reaction mixture was withdrawn and the proteins were precipitated by the addition of 0.1 ml of 4 *N* perchloric acid + 0.6 ml of water. After standing in an ice bath for at least one hour the solution was centrifuged. To 0.9 ml of the supernatant was added 0.2 ml of 4 *N* KOH and the light absorption of the alkaline solution at 300 mμ was measured against a blank, which consisted of phosphate buffer + enzyme and had been treated in exactly the same way as the sample. Under these conditions uracil had a molar extinction coefficient of $1.43 \cdot 10^3$. From this value the amount of uracil formed during the incubation could be calculated. In a parallel experiment enzyme, 50 μmoles of uridine and 100 μmoles of tris buffer, pH 7.4, were treated in the same way. Under these conditions (in the absence of added phosphate) the increases in light absorption at 300 mμ were much smaller, indicating that a true phosphorolysis of uridine was being measured (see Fig. 1).

Uridine kinase. The methods described previously[10] were used in most cases for the determination of this enzyme. The UMP-2-^{14}C formed was chromatographed on Dowex-2-formate and its total radioactivity was taken as a measure of the enzyme reaction.

In an alternative method paper chromatography was used for the separation of the products of the reaction. This procedure was sometimes used when the influence of the concentration of uridine on the reaction rate was being studied. For this purpose 7.5 μmoles of $MgSO_4$, 1.5 μmoles of ATP, 4.5 μmoles of PGA and enzyme were incubated at 37° for 15 min in a final volume of 0.30 ml together with different amounts of uridine-2-^{14}C. The reaction was stopped by the addition of 0.10 ml of 4 *N* PCA.

After centrifugation the supernatant was heated at 100° for 10 min to break pyrophosphate bonds. The cooled solution was neutralized to pH 5–7 with 4 *N* KOH and the $KClO_4$ formed was allowed to precipitate in an ice bath. Aliquots of the supernatant (0.005–0.03 ml) were subjected to descending paper chromatography on Whatman No. 1 filter paper. The solvent used was a modification of that used by PLESNER[18] (20 ml of 5 *M* ammonium acetate, pH 9, + 80 ml of saturated sodium tetraborate + 180 ml of ethanol + 0.5 ml of 0.5 *M* versene). Complete separation of uridine-5′-phosphate (R_F = *ca.* 0.18), uridine-3′-phosphate (R_F = *ca.* 0.40), uridine (R_F = *ca.* 0.48), and uracil (R_F = *ca.* 0.71) was achieved. The areas corresponding to each spot were eluted with water and ^{14}C-determinations performed on aliquots of the eluant in a Tracerlab Sc-18 windowless flow counter.

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Assuming a 100% recovery of the total radioactivity in the chromatogram the amounts of uracil and UMP formed could be calculated from the radioactivity in each fraction.

Catabolism of uracil. The work of CANELLAKIS[19] and FRITZSON[20] has clearly demonstrated that the formation of dihydrouracil is the rate-limiting step in the degradation of uracil to CO_2, NH_3 and β-alanine. The formation of $^{14}CO_2$ from uracil-2-^{14}C could, therefore, conveniently be taken as a measure of the capacity of the different enzyme preparations to degrade uracil.

In our experiments 1 μmole of uracil-2-^{14}C, 0.67 μmoles of TPN, 15 μmoles of isocitrate and different amounts of enzyme, pH 7.4, were incubated for 15 min at 37° in a final volume of 3 ml. Separate experiments showed that the amount of isocitrate dehydrogenase in the crude enzyme extracts was sufficient to maintain a satisfactory level of TPNH during the experiments. The incubations were carried out in stoppered 25 ml Warburg vessels containing 4 *N* KOH in the center well. At the end of the experiment 100 μmoles of $NaHCO_3$ was added as carrier and 4 *N* PCA was tipped in from the side arm. CO_2 was collected in the center well, precipitated as $BaCO_3$ and counted at infinite thickness in an end-window Geiger counter as described by SMITH AND REICHARD[21]. The amount of radioactive CO_2 liberated from uracil during the reaction could be calculated from the ^{14}C-activity of the $BaCO_3$ by means of an experimentally determined conversion factor.

RESULTS

Pyrimidine nucleoside phosphorylase

In preliminary experiments uridine was formed from uracil + inosine in the presence of phosphate according to the following reactions:

$$\text{Inosine} + \text{phosphate} \xrightarrow[\text{phosphorylase}]{\text{purine nucleoside}} \text{ribose-1-phosphate} + \text{hypoxanthine} \qquad (3)$$

$$\text{Ribose-1-phosphate} + \text{uracil} \xrightarrow[\text{phosphorylase}]{\text{pyrimidine nucleoside}} \text{uridine} + \text{phosphate} \qquad (4)$$

Reactions (3) and (4) are in all probability catalysed by two different enzymes[22,17] and the results given in Table I, are therefore, a measure of the rate-limiting step in the two reactions. Nevertheless, it was found that uridine was formed in all three tissues under the experimental conditions and that the reaction proceeded much slower in the mouse liver extract.

The spectrophotometric assay permitted a direct comparison of the rate of reaction (4) in the different extracts (Fig. 1). In the absence of phosphate little uracil was formed. The rate of the reaction in the presence of phosphate was linear only when very small amounts of uracil were formed. This type of behaviour has been found by FRIEDKIN AND ROBERTS[17] in the case of the phosphorylysis of thymidine and was shown to be due to a marked product inhibition of the reaction. The assay is therefore only semiquantitative. From the curves one can calculate that after 30 minutes the

TABLE I

FORMATION OF URIDINE-^{14}C FROM URACIL-^{14}C AND INOSINE THE PRESENCE OF PHOSPHATE

	Ascites tumour	*Rat liver*	*Mouse liver*
μmoles uridine formed	1.18	0.70	0.17
μmoles uracil recovered	15.0	11.1	11.9
% conversion	7.3	6.3	1.4
% recovery of total ^{14}C	81	59	60

In each experiment 50 μmoles of inosine, 20 μmoles of uracil-2-^{14}C (80,000 counts/min/μmole), 250 μmoles of phosphate buffer, pH 7.4, and enzyme from 500 mg of acetone powder were incubated in a volume of 6.4 ml at 37° for 30 min. After deproteinization and precipitation of $KClO_4$, the sample was deionized by passage through a Dowex-2 formate and a Dowex-50 H^+ column. The solution was evaporated *in vacuo* and subjected to starch chromatography[15].

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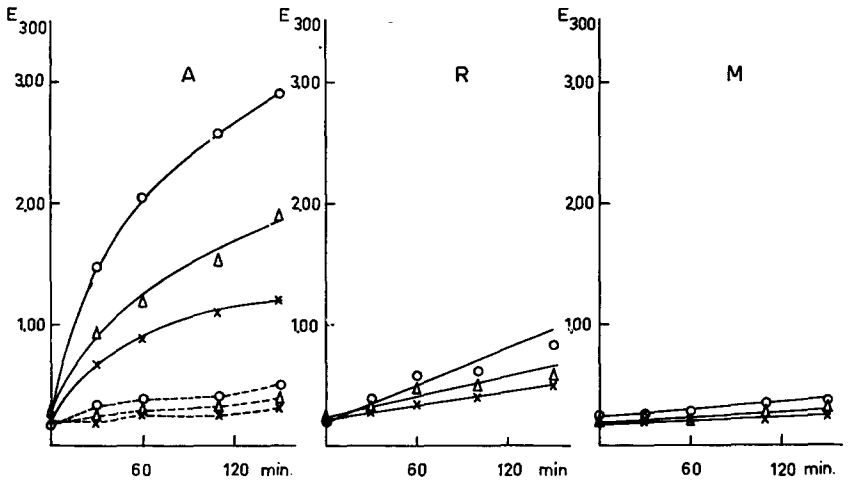

Fig. 1. Phosphorylytic cleavage of uridine. —×—×— = extract from 30 mg acetone powder; —△—△— = extract from 50 mg acetone powder; —○—○— = extract from 100 mg acetone powder. A = ascites tumour; R = rat liver; M = mouse liver. Broken lines = experiments without phosphate. For further explanation see EXPERIMENTAL.

following amounts of uracil (μmoles) were formed per mg of enzyme nitrogen* in the different extracts: ascites tumor: 2.2 (2.6, 2.2, 1.7); rat liver: 0.30 (0.45, 0.22, 0.22); mouse liver: 0.04 (0.06, 0.03, 0.03). In each case the first value given is the mean of the three values at different (increasing) enzyme concentrations given in the parentheses. The large differences between the various tissues were consistently observed in several other experiments.

*Uridine kinase***

In preliminary experiments using method 2 of our earlier paper[10] it was found that the kinase activity was about 5–10 times greater in acetone powder extracts of the ascites tumour than in those of rat liver. Mouse-liver extracts were 2–4 times more active than rat liver. The same general results were obtained when the "supernatant fractions" from homogenates of the tissues were used rather than acetone powder extracts. In the case of the supernatant fractions all activities were about 2–3 times higher on a nitrogen basis than in acetone powder extracts.

Figs. 2 and 3 represent an experiment analysed by method 1 of our previous paper[10]. Uridine and uracil were isolated from the first peak of Fig. 2 as described in the text to Table I.

The total amount of uridine phosphates synthesized was 8 times greater in the ascites tumour than in rat liver, while mouse liver occupied an intermediate position. In the ascites tumour most of the uridine phosphates were present as UTP, whereas in the liver extracts a large part was present as UMP. A high level of triphosphates in

* In all three tissues 100 mg of acetone powder correspond to *ca.* 2.5 mg enzyme-nitrogen in the extract.

** Uridine was not phosphorylated when AMP, UMP, CMP or phenyl phosphate was substituted for ATP, shoving the non-involvement of nucleoside phosphotransferase (G. BRAWERMAN AND E. CHARGAFF, *Biochim. Biophys. Acta*, 15 (1954) 549). Optimal results were obtained when ATP was continously regenerated from PGA, indicating the action of a kinase.

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tumour extracts has previously been found by SCHMITZ *et al.*[23]. The ATP level was well maintained in all three experiments (Fig. 2).

A summary of the amounts of all radioactive compounds is given in Fig. 3. In all three experiments there was some uridine left at the end of the incubation.

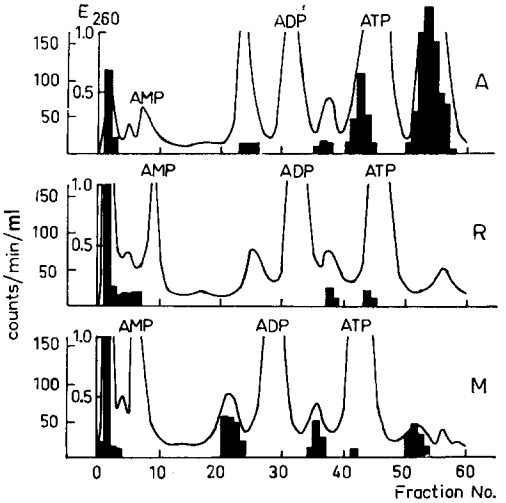

Fig. 2. Formation of uridine phosphates from uridine. In each experiment 20 μmoles of ATP, 50 μmoles of PGA, 100 μmoles of $MgCl_2$, 3.04 μmoles of uridine-2-^{14}C, 150 μmoles of tris buffer pH 7.4 and enzyme from 300 mg of acetone powder were incubated in a volume of 4.2 ml at 37° for 15 min. Analysis according to method 1[10]. Continuous curve = E_{300}. Stepped curve = counts/min/ml.

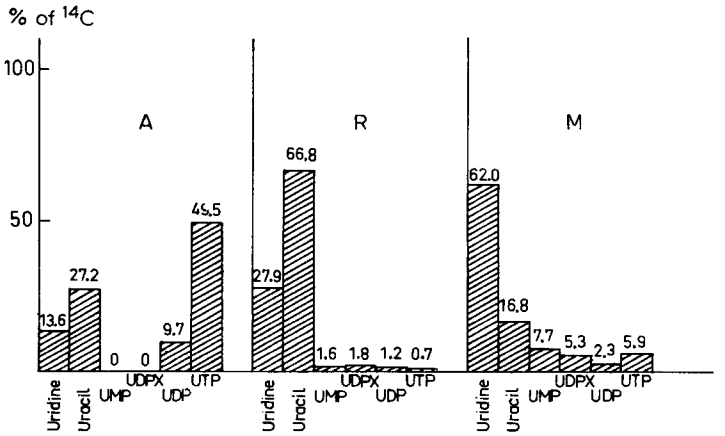

Fig. 3. Distribution of different uracil compounds from Fig. 2. For explanation of UDP-X, see ref. [8].

When the formation of uridine phosphates was studied at increasing uridine concentrations the results given in Fig. 4 were obtained. In these experiments method 2 was used[10], in which all uridine phosphates formed are analysed as UMP. In a similar experiment the formation of CMP from cytidine was studied in ascites tumour extracts. Saturation of the enzyme required high concentrations of uridine (*ca.* 10^{-2} *M*), but was achieved at much lower concentrations of cytidine (less than $5 \cdot 10^{-4}$ *M*).

It was possible that at low uridine concentrations all substrate was converted to uracil by the action of pyrimidine nucleoside phosphorylase. Therefore, the amounts of uracil and UMP formed at different uridine concentrations were determined with the paper chromatographic method described in the experiment part. Fig. 5 shows

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that the need for a high uridine concentration for optimal UMP synthesis was not due to the rapid disappearance of uridine. The curves again also demonstrate the great difference in both kinase and phosphorylase activities of the three extracts.

From Fig. 5 the following values could be calculated. *Uridine kinase* (μmoles of UMP formed during 15 min per mg enzyme nitrogen): ascites tumour: 3.5; rat liver:

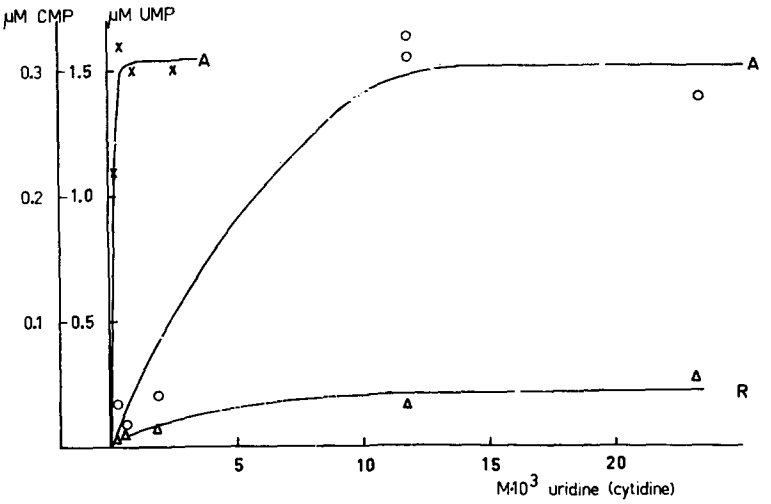

Fig. 4. Dependence of UMP and CMP formation on substrate concentration. *UMP-formation*: Experimental conditions as in Fig. 2 though extracts from 200 mg of rat liver powder and 50 mg of ascites tumour powder respectively were used at different uridine concentration. *CMP-formation*: The paper-chromatographic method described in the experimental part was used. Absolute amounts of UMP and CMP formed are not comparable since different preparations of ascites tumour extracts were used. —△—△— and —○—○— = UMP formation; —×—×— = CMP formation.

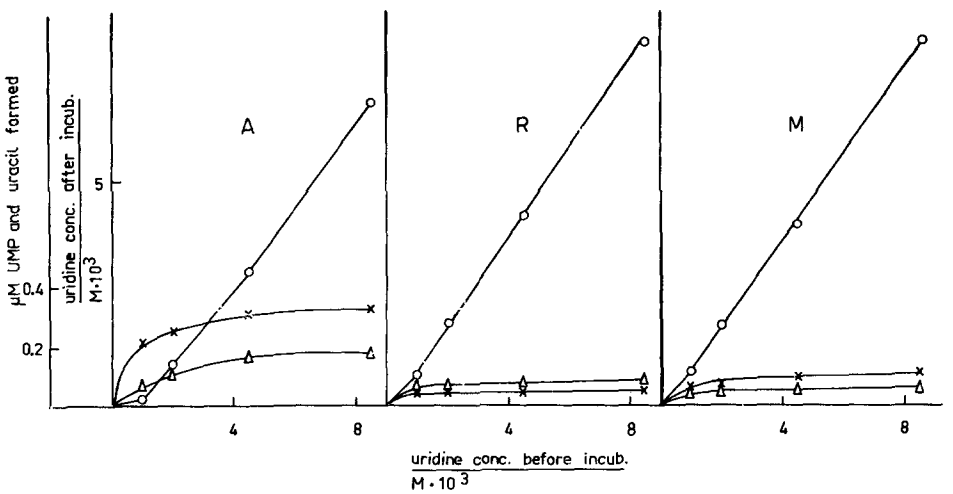

Fig. 5. Pyrimidine nucleoside phosphorylase and kinase activity at different uridine concentrations. The substrate composition was that described under EXPERIMENTAL (paper-chromatographic method); the enzyme extracts were from 25 mg of rat liver and mouse liver acetone powder, respectively and from 3.4 mg of ascites tumour acetone powder. ○ ○ = uridine; —×—×— = UMP; —△—△— = uracil.

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0.08; mouse liver: 0.16. *Pyrimidine nucleoside phosphorylase* (μmoles of uracil formed during 15 min per mg enzyme nitrogen): ascites tumour: 1.9; rat liver: 0.13; mouse liver: 0.06. All values are calculated at optimal uridine concentrations.

Catabolism of uracil

CANELLAKIS[19] and GRISOLIA *et al.*[24] have shown that the formation of dihydrouracil from uracil (the first step in the catabolism of uracil) is dependent on the presence of TPNH. In confirmation of the results of these authors we found that the addition of a TPNH-regenerating system to a crude non-dialysed extract of rat-liver acetone powder increased the formation of $^{14}CO_2$ from uracil-2-^{14}C about 7 times. When comparing the enzyme activity of different extracts it was, therefore,

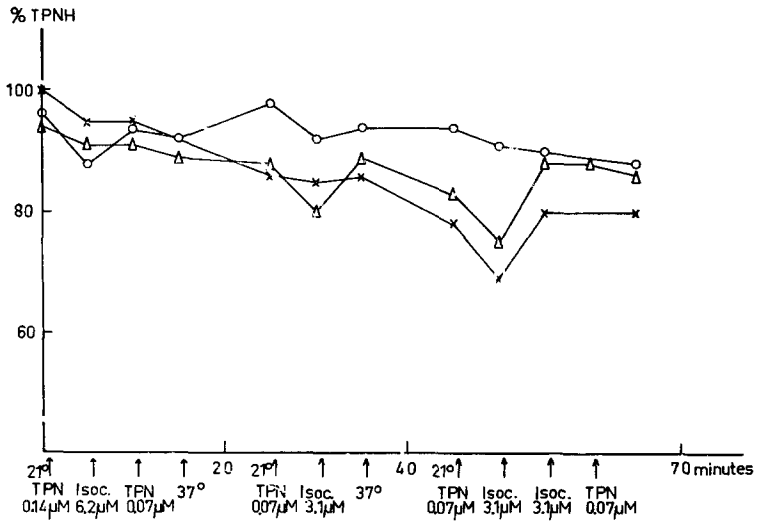

Fig. 6. Maintenance of TPNH level. From the beginning each Beckman cell contained 1 μmole of uracil, 0.35 μmole of TPN, 15 μmoles of isocitrate, 100 μmoles of $MgCl_2$ and *ca.* 1.5 mg of enzyme nitrogen ("supernatant fraction", pH 7.4). Incubation and additions as indicated in the figure. Readings at 340 mμ were taken against a blank without TPN. 100% TPNH was calculated from the molar extinction coefficient of TPNH at 340 mμ = $6.2 \cdot 10^3$. —⊙—⊙— = ascites tumour; ---△—△— = rat liver; ---×—×— = mouse liver.

of the utmost importance to ensure adequate TPNH levels in all cases studied. In our experiments this was achieved by the addition of isocitrate + TPN to the extracts. The light absorption at 340 mμ was taken as a measure of the amount of TPNH present in the system (Fig. 6). Comparable results were obtained in all the three tissue extracts studied.

In Table II a comparison is given of the breakdown of uracil in extracts from the three tissues. Both acetone powder extracts and supernatants from tissue homogenates were used. In these and in several other experiments no activity could be demonstrated in the ascites tumour. Rat liver was more active than mouse liver. In confirmation of CANELLAKIS' results[19] we found that the activity of the supernatant fraction from liver homogenates was considerably higher than that of acetone powder extracts from whole liver.

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TABLE II

FORMATION OF $^{14}CO_2$ FROM URACIL-2-^{14}C

	Counts/min ($Ba^{14}CO_3$ at infinite thickness)					
	Ascites tumour		*Rat liver*		*Mouse liver*	
ml Enzyme	*Acetone powder*	*Supernatant*	*Acetone powder*	*Supernatant*	*Acetone powder*	*Supernatant*
0.5	5	0	80	186	33	52
1.0	2	0	146	364	59	147
1.5	–	0	173	483	70	288

Conditions as described under EXPERIMENTAL. One ml of enzyme solution contained (mg protein nitrogen): Ascites tumour: 5.8 (acetone powder) and 0.55 (supernatant fraction); rat liver: 6.5 (acetone powder) and 3.0 (supernatant fraction); mouse liver: 7.9 (acetone powder) and 2.1 (supernatant fraction).

It has been reported that the addition of ATP increases the formation of CO_2 from uracil[25], but we could not observe such an effect under our experimental conditions.

From the data of Table II (lowest enzyme concentrations) and an experimentally determined conversion factor it can be calculated that the following amounts of CO_2 were formed per mg of protein nitrogen during 15 minutes: Rat liver: 0.0037 μmoles (acetone powder) and 0.018 μmoles ("supernatant fraction"); mouse liver: 0.0013 μmoles (acetone powder) and 0.0074 μmoles ("supernatant fraction").

DISCUSSION

The aim of our investigation was to compare the amount of enzymes, involved in uracil catabolism and anabolism, present in some tissues that showed large differences in their capacity to utilize uracil for polynucleotide synthesis *in vivo*. The two extremes chosen were rat liver, which incorporates labelled uracil to a very limited extent[1], and the Ehrlich ascites tumour, which utilizes labelled uracil about as well as orotic acid[7]. Mouse liver, which occupies an intermediate position, was also included in our investigation. We should like to emphasize that in this investigation we did not intend to compare a cancerous tissue with its homologous normal counterpart; the ascites tumour originated as a mammary cancer.

Our determinations of enzyme concentrations in extracts of rat liver and ascites tumour clearly demonstrate the large difference between the two tissues as regards the amounts of enzymes involved in both the catabolic and anabolic metabolism of uracil. In agreement with earlier workers[19,25], we found that uracil-2-^{14}C was degraded to $^{14}CO_2$ by the liver preparations. No such activity was found in the tumour extracts. The levels of both uridine phosphorylase and uridine kinase, however, were considerably higher in tumour extracts than in rat liver extracts. This was especially pronounced for uridine kinase, which on a protein basis consistently showed 5–30 times higher activity in the tumour. On the whole, this enzyme pattern is more favourable to UMP synthesis in the ascites tumour than in rat liver.

It was, furthermore, evident from our data that the limiting enzyme reaction influencing UMP synthesis in rat liver was uridine kinase. The breakdown of uracil

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was a relatively slow process and, therefore, influenced uracil metabolism only to a small extent*.

It is also interesting that the affinity of the uridine kinase for uridine is quite low, saturation being achieved at approximately 0.01 *M* concentration. This explains the greatly increased incorporation of labelled uracil and uridine** into RNA with increasing precursor concentrations, which has been observed, previously, both in the rat *in vivo*[4] and in rat liver slices[6]. The fact that optimal nucleotide formation was obtained at a much lower concentration of cytidine, at least partly explains why in earlier work labelled cytidine was a much more effective precursor for RNA pyrimidines than uridine, when the two nucleosides were administered in equimolar amounts to rats[26].

Mouse liver showed a somewhat smaller capacity to degrade uracil than did rat liver. The level of uridine kinase was intermediate between these of the tumour and in rat liver. Uridine phosphorylase activity was considerably lower than in the other two tissues investigated and probably represented the limiting reaction in UMP formation.

It is clear that the pattern of the enzymes involved in uracil metabolism in the Ehrlich ascites tumour favours anabolic reactions. Possibly this represents an enzymic adaptation of the tumour, correlated with the much increased rate of polynucleotide synthesis that occurs in this rapidly growing tissue. We found that the ascitic serum surrounding the tumour cells contained uracil*** (*ca.* 2–3 μmoles/100 ml). This uracil might originate from host tissues and be utilized by the tumour for nucleotide synthesis.

According to our point of view uracil is re-utilized for polynucleotide synthesis and not synthesized *de novo* from small molecules. FINK *et al.*[27], GRISOLIA *et al.*[21], CANELLAKIS[19] and FRITZSON[20] have established a series of reactions by which CO_2, NH_3 and β-alanine are formed from uracil via dihydrouracil and β-ureidopropionic acid. FRITZSON[20] has demonstrated that the conversion of uracil to dihydrouracil is not reversible *in vitro* and that the final hydrolytic cleavage of β-ureidopropionic acid is irreversible[21]. The original evidence for the formation of β-ureidopropionic acid from β-alanine + carbamyl phosphate seems now to be of doubtful significance§.

Furthermore, the earlier experiments of LAGERKVIST *et al.*[7] demonstrated that labelled β-alanine, β-ureidopropionic acid or dihydrouracil were not utilized for polynucleotide synthesis by the Ehrlich ascites tumour. Dilution experiments with the same non-labelled compounds or non-labelled uracil and $^{15}NH_4Cl$ also gave negative results. In conclusion all the evidence cited above speaks against the concept of a *de novo* synthesis of free uracil.

* So far uracil catabolism has only been demonstrated in mammalian liver and microbial systems (R. M. FINK, R. E. CLINE AND H. M. C. KOCH, *Federation Proc.*, 13 (1954) 207) and it seems possible that in the mammal uracil catabolism is restricted to the liver. In rat intestine no degradation of uracil was found by CANELLAKIS[19]. Nevertheless, the utilization of uracil for polynucleotide synthesis was much smaller than that of orotic acid in this tissue[4]. Uracil catabolism could not be the reason for this and it seems more likely that the anabolic reactions involving uracil were deficient.

** This applies also to the corresponding experiments with labelled UMP, since the available evidence indicates that mononucleotides enter liver cells only after dephosphorylation.

*** The uracil was prepared by starch chromatography of the deproteinized ascites serum, which had been deionized by passage through Dowex-2 and Dowex-50. Uracil was identified from its position on the starch chromatogram, by paper chromatography and from its spectrum.

§ S. GRISOLIA, personal communication.

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It seems that several purine and pyrimidine analogues[28-32] exert their carcinostatic actions by being incorporated into polynucleotides in lieu of "natural" purines or pyrimidines. A synthetic mechanism such as the formation of UMP from uracil in this ascites tumour would seem to be especially suited for this type of chemotherapeutic attack, since it represents not the ordinary *de novo* pathway of pyrimidine biosynthesis but rather an auxiliary mechanism which is utilized to a high degree by this rapidly growing tumour.

SUMMARY

The content of enzymes involved in uracil metabolism (pyrimidine nucleoside phosphorylase, pyrimidine nucleoside kinase and enzymes of uracil catabolism) in extracts from rat liver, mouse liver and the Ehrlich ascites tumour was studied.

Much higher levels of enzymes involved in anabolic reactions were found in ascites tumour extracts than in liver, whereas the enzymes of uracil catabolism which were present in rat and mouse liver were absent from the tumour. Especially the relatively high concentration of pyrimidine nucleoside kinase in the tumour extracts probably explains the better utilization of free uracil for polynucleotide synthesis in this tissue as compared to rat liver.

Pyrimidine nucleoside kinase needed a high concentration of uridine (*ca.* 0.01 *M*) for optimal synthesis of nucleotides, while a considerably lower concentration of cytidine (less than 0.0005 *M*) was sufficient. This partly explains the much better utilization of cytidine for polynucleotide synthesis as compared to uridine.

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