THE EFFECT OF URIDINE ADMINISTRATION ON THE ACTIVITIES OF URIDINE AND DEOXYURIDINE PHOSPHORYLASES OF REGENERATING RAT LIVER

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Enzyme systems for the incorporation of uracil into RNA have been demonstrated in normal and regenerating rat liver (Canellakis, 1957b), tumor cells (Canellakis, 1957b; Reichard and Skold, 1958), and other mammalian tissues (Rutman, Cantarow and Pasckis, 1954; Heidelberger, Liebman, Harbers and Bhargava, 1957). Two of the enzymes concerned in this incorporation are uridine phosphorylase and uridine kinase (Canellakis, 1957a; Reichard and Skold, 1957, 1958; Pontis, Degerstedt and Reichard, 1961). In both regenerating rat liver and Ehrlich ascites tumor cells uracil is converted to nucleotides and incorporated into RNA more rapidly than in normal, resting tissues (Canellakis, 1957b; Reichard and Skold, 1958). This increased rate of utilization of uracil for RNA synthesis by rapidly dividing tissues has been explained in part by a reduction in the capacity of these tissues to degrade uracil (Canellakis, Jaffe, Mantsavenos and Krakow, 1959; Reichard and Skold, 1958). The purpose of this communication is to report that an injection of uridine can cause a doubling of the uridine phosphorylase activity and an increase in the deoxyuridine phosphorylase activity of rat liver, 48 hours after partial hepatectomy.

Under ether anesthesia male Holtzman rats of between 300 and 440 grams were partially hepatectomized. In every case, two lateral lobes of liver were removed. The animals were fed commercial rat pellets ad libitum prior to and after the operation. Forty-eight hours post-operatively, six rats were injected, intraperitoneally with 150 mg. of uridine per 100 gm. of

body weight, and six control rats were injected with 0.9 per cent sodium chloride. Five hours later the animals were decapitated, the livers were perfused *in situ* with ice-cold 0.9 per cent saline, and removed. The livers were homogenized in five volumes of ice-cold 0.05 M potassium phosphate buffer at pH 7.2 containing 5 mM mercaptoethanol and the homogenates were dialyzed for 18 hours in 100 volumes of this buffer. Uridine and deoxyuridine phosphorylase activities of each homogenate were determined by a modification of the method of Canellakis (Canellakis, 1957b; Duke and Yamada, 1962). A unit of enzyme activity is defined as the amount that liberates 1 μmole of uracil per hour under the standard assay conditions. Specific activity is expressed in units per mg. of protein. Protein was measured by the method of Lowry et al (Lowry, Rosebrough, Farr and Randall, 1951).

In some experiments five times the concentration of dialyzed homogenate and substrate were incubated under the standard assay conditions. The products of the reaction were identified by two-dimensional paper chromatography (Carter, 1950). In all tests, no uridylic acid could be detected. The dialyzed homogenates were also tested for dihydrouracil dehydrogenase activity (Canellakis, 1956). If $TPNH_2$ was omitted from the incubation mixture, the conversion of uracil to dihydrouracil could not be detected.

The data in Table I indicate that considerable uridine phosphorylase activity is present in regenerating rat liver five hours after uridine injection. The mean specific activity of the uridine phosphorylase of the livers of rats given uridine was about twice that of the livers of control rats ($P < 0.001$). The enzyme levels of the control rat livers were rather constant over the weight range studied.

It is apparent from Table II that the activity of deoxyuridine phosphorylase of regenerating rat liver is also increased after uridine injection ($P < 0.001$). In every animal studied, whether injected with uridine or with 0.9 per cent sodium chloride, the liver deoxyuridine phosphorylase was always more active than the uridine phosphorylase.

TABLE I

The Effect of Uridine Administration on Uridine Phosphorylase Activity of 48 Hour Regenerating Rat Liver

Saline Injected			Uridine Injected		
Body Weight (gm.)	Wet Weight of Liver (gm.)	Uridine Phosphorylase (units/mg. protein)	Body Weight (gm.)	Wet Weight of Liver (gm.)	Uridine Phosphorylase (units/mg. protein)
296	6.6	0.101	309	7.7	0.329
340	6.5	0.107	366	11.5	0.304
409	9.8	0.115	408	6.2	0.204
420	11.0	0.130	412	8.5	0.216
430	9.1	0.099	432*	9.1	0.189
440	5.7	0.106	434	6.0	0.279
Mean $\pm$ SEM =	389.2 $\pm$ 23.6	0.110 $\pm$ 0.0147	393.8 $\pm$ 19.8		0.254 $\pm$ 0.0238

* Injected with 100 mg. of uridine per 100 gm. body weight.

TABLE II

The Effect of Uridine Administration on Deoxyuridine Phosphorylase Activity of 48 Hour Regenerating Rat Liver

Saline Injected			Uridine Injected		
Body* Weight (gm.)	Wet Weight of Liver (gm.)	Deoxyuridine Phosphorylase (units/mg. protein)	Body* Weight (gm.)	Wet Weight of Liver (gm.)	Deoxyuridine Phosphorylase (units/mg. protein)
296	6.6	0.196	309	7.7	0.298
340	6.5	0.170	366	11.5	0.283
409	9.8	0.168	408	6.2	0.218
420	11.0	0.169	412	8.5	0.349
430	9.1	0.215	432**	9.1	0.265
440	5.7	0.181	434	6.0	0.334
Mean $\pm$ SEM =		0.183 $\pm$ 0.008			0.291 $\pm$ 0.0195

* These are the same animals as in Table I.

** Injected with 100 mg. of uridine per 100 gm. body weight.

Preliminary experiments indicate that the uridine phosphorylase activity of regenerating rat liver is also increased by the administration of azauridine (Yamada, 1962) or cortisol (Duke and Yamada, 1962).

It has been postulated that the synthesis of RNA of tissues can be regulated by changes in the activities of the synthetic and degradative pathways. In support of this view it was found that rapidly dividing tissues degrade uracil less rapidly than do normal tissues, and utilize more uracil for RNA synthesis (Canellakis, et al, 1959; Canellakis, 1957b; Reichard and Skold, 1958). Our work suggests that another control mechanism may also operate in rapidly dividing tissues. It has been shown that the injection of uridine increased the activities of both uridine and deoxyuridine phosphorylases of regenerating rat liver.

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