Uridine Kinase Activities in Developing, Adult and Neoplastic Rat Tissues

By Annemarie HERZFELD and Suzanne M. RAPER

Cancer Research Institute of the New England Deaconess Hospital and the Department of Biological Chemistry, Harvard Medical School, Boston, MA 02215, U.S.A.

(Received 18 April 1979)

Uridine kinase activities were found chiefly in the soluble fractions of rat tissues. In normal adults the activities ranged from 13 munits/g in skeletal muscle to 178 munits/g in colon. Enzyme activities in several rat neoplasms were significantly higher (e.g. in a fibrosarcoma, mammary carcinoma, renal carcinoma, pancreatic carcinoma and lymphocytic lymphoma, but not in a fast-growing Morris hepatoma). The activities were not related to tumour growth rates or sizes. In normal foetal liver, lung, brain, heart and kidney, uridine kinase concentrations equalled or exceeded those in the adult homologous tissue, but maximal activities in liver were reached 3–5 days *post partum*. In suckling rats the intestinal activity decreased substantially immediately after birth and normally did not rise again until late in the third postnatal week. Premature upsurges could be evoked by an injection of cortisol or by starvation of the pups overnight. Pancreatic activity was absent from 1-day-old rats, and only about 5% of the adult activity was reached by day 20; adult activities were attained rapidly after weaning. In pancreas, precocious formation of uridine kinase was elicited by overnight starvation of 2-week-old rats.

The phosphorylation of uridine by uridine kinase (EC 2.7.1.48) is considered an important step in the salvage pathway for the formation of RNA (Reichard & Sköld, 1958; Canellakis *et al.*, 1959; Sköld, 1960a). The enzyme has been of special interest because of its putative rate-limiting role in the pathway and its reported close correlation with rates of tissue growth (normal and neoplastic) (Reichard & Sköld, 1958; Krystal & Webb, 1971). It is also thought to be the sensitive step in the actions of some chemotherapeutic agents (e.g. 5-azacytidine and halogenated uridines) (Greenberg *et al.*, 1977). Attention has focused on the investigations of the enzyme in partially purified preparations from rodent neoplasms (Sköld, 1960a; Krystal & Webb, 1971), from regenerating rat liver (Krystal & Webb, 1971) and from phytohaemagglutinin-stimulated lymphocytes (Greenberg *et al.*, 1977), all of which have high concentrations of uridine kinase. The differential elution of two peaks of soluble uridine kinase activity from Sepharose 6B columns (Krystal & Webb, 1971; Greenberg *et al.*, 1977) has suggested that the enzyme occurs in two isoenzymic forms whose predominance shifts in the course of maturation or neoplastic transformation (Krystal & Webb, 1971; Keefer *et al.*, 1974).

We have studied uridine kinase in crude extracts of adult, developing and neoplastic rat tissues. We have altered the normal physiological state of developing rats by cortisol injections or starvation to determine if those stimuli might prompt the accumulation of uridine kinase. The enzyme was

induced by cortisol in the intestine of rats younger than 12 days, and its accumulation was triggered in small intestine and pancreas throughout the suckling period by overnight starvation. Neither treatment changed the enzyme amounts substantially in liver. The regulation of uridine kinase therefore appears to be tissue-specific and age-dependent. Its sensitivity to the two treatments in liver and intestine contrasts with that of thymidine kinase (EC 2.7.1.75), the critical enzyme in the salvage pathway for DNA synthesis (Machovich & Greengard, 1972).

Experimental

Adult tissues were obtained from 60–90-day-old male isogenic Kx (New England Deaconess Hospital breeding colony) or CDF (Charles River Breeding Laboratory, Wilmington, MA, U.S.A.) rats. Foetuses (from time-mated CDF dams) and immature rats were from the CDF strain. Animals were weaned at 23 days to Purina rat chow and water *ad lib*. Tumours were implanted into the flanks of male rats 40–60 days old. The provenance of the fibrosarcoma RNC 254 (in Kx rats), the mammary carcinoma DMBA 5 A (in CDF rats), and the Morris hepatoma 7777 and renal carcinoma MK-1 (in Buffalo rats) has been described (Herzfeld & Greengard, 1972, 1977; Herzfeld *et al.*, 1978). The lymphocytic lymphoma arose spontaneously in the non-irradiated partner of a parabiotic pair (S. Warren, unpublished work) and has been carried by us for ten transplant generations in male Kx rats, and the pancreatic

carcinoma, maintained by us in CDF rats, was a gift from Dr. J. K. Reddy and Dr. M. S. Rao of Chicago.

Postnatal rats were injected intraperitoneally with 2.5 mg of cortisol/100 g body wt. in 0.9% NaCl (cortisol acetate; Merck, Sharp and Dohme, West Point, PA, U.S.A.) and either returned to their dams or isolated without food at 30°C for 18 h. Control littermates were kept with their dams or deprived of food at 30°C for 18 h ('starved' rats).

Adult intestine was divided into duodenum (from pylorus to ligament of Treitz), jejunum (5 cm distal from ligament of Treitz), ileum (5 cm proximal to caecum) and colon (5 cm distal from caecum). The segments were rinsed with distilled water before homogenization.

Tissues, freshly excised, were suspended in 9 vol. of cold 0.15 M-KCl, disintegrated in glass-Teflon homogenizers and centrifuged at 100 000 g for 30 min. The supernatant fractions and the pellets, resuspended to the original volume in 0.5% Triton X-100 in water, were assayed immediately for enzyme activities. Particle fractions, not suspended in Triton, had lower activities; the soluble activity was not diminished in the presence of the detergent.

Uridine kinase activity measurements were based on the procedure of Krystal & Webb (1971). Stock uridine solutions (1.25 mM, containing 0.4 mCi/mmol), 0.05 M-ATP, pH 7.4, 0.5 M-Tris/HCl buffer, pH 7.4, and 0.1 M-MgCl₂ were kept frozen or refrigerated for up to 1 month. Reaction mixtures, in total volumes of 0.3 ml, contained 0.1 ml of uridine solution (final concn. 0.42 mM), 4.2 mM-ATP, 8.3 mM-

MgCl₂, 83.3 mM-Tris/HCl buffer, pH 7.4, and 0.05 ml of enzyme preparation (equivalent to up to 5 mg of tissue). Tubes were incubated at 37°C for 20 min and then 50 µl samples were spotted on DEAE-cellulose paper discs (Whatman DE 81) (Machovich & Greengard, 1972), washed for 10 min in 1 mM-ammonium formate, for 5 min in water and for 5 min in 95% (v/v) ethanol. Discs were dried and counted for radioactivity in 10 ml of Aquasol in a Packard scintillation counter at 75% efficiency. Blanks, incubation mixtures from which nucleoside triphosphates were omitted, were subtracted from experimental values. Complete samples (without incubation) or enzyme-free reaction mixtures gave values similar to the blanks that were used. Enzyme activities are expressed as munits (nmol of uridine phosphorylated/min) per g of tissue.

[2-¹⁴C]Uridine (50 mCi/mmol), [5-³H]cytidine (25 Ci/mmol) and Aquasol were obtained from New England Nuclear Corp., Boston, MA, U.S.A. Nucleosides, nucleotides and Triton X-100 were bought from Sigma Biochemical Co. (St. Louis, MO, U.S.A.), Calbiochem (La Jolla, CA, U.S.A.) or Boehringer-Mannheim Co. (Indianapolis, IN, U.S.A.). Deoxyfluorouridine was obtained from Roche Laboratories (Nutley, NJ, U.S.A.). Other chemicals used were reagent grade.

Results

The uridine kinase reaction was linear with incubation time at 37°C for at least 30 min and with the enzyme concentration between 1.0 and 6.0 mg equivalent of tissue/0.3 ml reaction mixture. When

Table 1. *Alternative substrates and additions of pyrimidine nucleosides to the uridine kinase reaction*
Samples of tissue extracts were incubated with 0.42 mM-substrate ([¹⁴C]uridine or [³H]cytidine) under the usual assay conditions; to test for the inhibition by other nucleosides, some reaction mixtures contained 0.42 mM-non-radioactive cytidine or thymidine (with [¹⁴C]uridine as substrate) or uridine (with [³H]cytidine as substrate). Enzyme activities, in munits/g of tissue, are shown as averages of two determinations (without S.D.) or as means ± S.D. when tissues from more than three rats were analysed.

		Enzyme activity (munits/g)				
		^[14C] Uridine			^[3H] Cytidine	
		None	Cytidine	Thymidine	None	Uridine
Additions ...						
Adult						
Liver		27.1 ± 2.7	14.4	27.3	6.8	3.9
Kidney		94.1 ± 12.7	45.2	—	14.3	7.7
Brain (particulate fraction)		28.1 ± 5.2	12.9	19.4	7.7	0.08
Brain (soluble fraction)		48.4 ± 10.5	23.2	48.8	7.7	7.2
Spleen		137.0 ± 21.0	61.7	—	18.7	11.8
Lung		63.4	26.6	—	—	—
Jejunum		120.0	48.0	—	—	—
Pancreas		76.3	30.5	—	—	—
Foetal						
Liver		75.5	35.5	—	—	—
Brain (particulate fraction)		42.4	19.9	—	—	—
Brain (soluble fraction)		89.0	42.7	—	—	—

samples were incubated at 37°C, the reaction rate was about twice that at 25°C. The reaction required Mg^{2+} and ATP or GTP as an alternative phosphate donor. CTP could not replace the purine nucleoside triphosphates. Activities were routinely measured under assay conditions in which only the amount of enzyme limited the reaction rate. In neither liver nor spleen was more than 5–8% of the total activity associated with particles; the particle fraction was not analysed in detail in those tissues. The cytosolic enzyme from adult rat liver and spleen was half-saturated with 0.5 mM-ATP (at 0.42 mM-uridine) and with 0.05 mM-uridine (at 4.2 mM-ATP). Both apparent K_m values agree with those reported by Sköld (1960b) for mouse Ehrlich ascites cells. Only the enzyme associated with fibrosarcoma particles was inhibited by ATP concentrations greater than 5 mM or uridine concentrations above 0.5 mM.

Soluble uridine kinase from jejunum and lung and the particulate brain enzyme exhibited similar

specificity for uridine (50–60% inhibition by equimolar cytidine and 25% of the reaction rate when 0.42 mM- $[^3H]$ cytidine replaced $[^{14}C]$ uridine as substrate) as the soluble enzyme from other adult tissues (Table 1). Thymidine, equimolar to the uridine added, inhibited only the enzyme from brain particles (Table 1). Uridine, when added to a reaction mixture containing $[^3H]$ cytidine as substrate, completely halted the phosphorylation of cytidine by particles from adult brain, but did not diminish that reaction when the soluble brain extract was the enzyme source. In the soluble fractions of other tissues the phosphorylation of cytidine was diminished by 37–46% in the presence of uridine (Table 1).

Heating the soluble uridine kinase from adult spleen and brain to 50°C for 5 min diminished the activity by 68% in the spleen and by over 82% in the brain. Inactivation of the particulate brain enzyme was 69% after 5 min at 50°C. When the preparations were kept at 50°C for 20 min, spleen activity was

Table 2. *Distribution of uridine kinase in rat tissues*

Values are means ($\pm$ S.D.) of results from the numbers of animals (or litters) shown in parentheses. The fibrosarcoma and lymphoma were grown in male Kx rats, the mammary carcinoma and pancreatic carcinoma in male CDF rats and the renal and hepatic Morris tumours in male Buffalo rats. All adult tissues were taken from male rats. Unless otherwise specified, the activities were those found in the soluble fraction after centrifugation of 0.15 M-KCl homogenates at 100000g for 30 min.

	Enzyme activities (munits/g)	
	Adult	Foetal (20–21 days gestation)
Normal tissues		
Thymus	171.0 (1)	
Spleen	140.0 $\pm$ 21.2 (7)	
Intestine: duodenum	138.0 (1)	
jejunum	130.0 $\pm$ 23.0 (5)	77.0 $\pm$ 19.0 (3 litters)
ileum	124.0 $\pm$ 15.0 (4)	
colon	178.0 $\pm$ 34.0 (4)	
Kidney: Kx rats	100.0 $\pm$ 9.8 (7)	113.0 $\pm$ 8.0 (3 litters)
Buffalo rats	74.0 $\pm$ 3.8 (3)	
Pancreas	98.0 $\pm$ 16.0 (5)	0 (2 litters)
Lung	68.0 $\pm$ 15.0 (6)	91.0 $\pm$ 14.0 (4 litters)
Brain: soluble fraction	54.0 $\pm$ 11.0 (5)	97.0 $\pm$ 2.0 (4 litters)
particulate fraction	32.0 $\pm$ 3.8 (5)	40.0 $\pm$ 7.3 (4 litters)
Liver: Kx or CDF rats	31.2 $\pm$ 5.2 (12)	47.0 $\pm$ 8.0 (5 litters)
Buffalo rats	18.2 $\pm$ 3.5 (3)	
host (Kx rats)	24.3 $\pm$ 1.9 (6)	
host (CDF rats)	23.5 $\pm$ 0.9 (3)	
host (Buffalo rats)	18.7 $\pm$ 3.1	
Heart	18.0 (1)	61.5 (1 litter)
Skeletal muscle	13.5 (1)	
Neoplastic tissues		
Monocytic lymphoma RNC 290	479 $\pm$ 52 (3)	
Fibrosarcoma RNC 254	390 $\pm$ 32.0 (5)	
Lymphocytic lymphoma RNC 314	337 $\pm$ 15.0 (4)	
Pancreatic carcinoma (Reddy & Rao, 1977)	244 $\pm$ 40.0 (4)	
Mammary carcinoma DMBA 5 A	277 $\pm$ 27.0 (3)	
Renal carcinoma MK 1	210 $\pm$ 11.0 (3)	
Morris hepatoma 7777	59.5 $\pm$ 7.5 (3)	

essentially the same as after 5 min of heating (70% loss), whereas both fractions of brain were totally inactivated.

In adult liver, kidney and lung, foetal liver and lung and fibrosarcoma, virtually all the uridine kinase activity was localized in the cytosolic cell fraction. A small residue of activity was observed in the fractions sedimenting at 600g from lung (adult and foetal), kidney and fibrosarcoma. Nearly half of the uridine kinase activity in foetal brain was associated with particles; in adult brain about 30% of the total activity was distributed equally between the mitochondrial and microsomal fractions. Attempts to dissociate the particulate activity from brain particles by hypo-osmotic washing released only a small portion of it and left over 21% still tightly bound to particles.

The distribution of uridine kinase in normal adult and foetal tissues and in some transplanted neoplasms of the rat is widespread (Table 2). All of the tissues that we have tested so far contained some uridine kinase activity, but, as expected, those tissues undergoing continuous cell renewal (e.g. thymus, spleen and intestine) exhibited the highest enzyme activities. Except in intestine and pancreas, the activities in tissues during late gestation were higher than in the adult (see also Figs. 1–3). The relatively high activities in the pancreatic carcinoma (Reddy & Rao, 1977) illustrate the unusual instance of an enzyme at high

concentrations in a neoplastic tissue but undetectable in the normal foetal one. As expected, most tumours (except for hepatoma 7777) had higher uridine kinase activities than normal adult tissues. The growth rates of the tumours with especially high uridine kinase activities differed substantially: their doubling times ranged from 1.5 to 20 days when calculated by changes in volume (Herzfeld & Knox, 1972). The relatively low activities in the Morris hepatoma 7777 (with a doubling time of less than 2.5 days) were thus surprising. Such comparatively low uridine kinase activity in the hepatoma invites contrast with the high thymidine kinase activity in the same tissue (Machovich & Greengard, 1972; Herzfeld & Greengard, 1977). It remains to be determined in additional host tissues if the lowered uridine kinase activities in the livers of some tumour hosts (20–40% below those of normal livers) (Table 2) illustrate a significant systemic effect of tumour-bearing. Such decreases in host livers in the activities of an enzyme that is high in foetal tissues and in tumours are rare in our experience (Herzfeld & Greengard, 1972, 1977; Herzfeld *et al.*, 1978); we have noted a single exception in pyrroline-5-carboxylate reductase (EC 1.5.1.12), high in foetal liver, which also was diminished in most host livers (Herzfeld & Greengard, 1977).

The developmental formations of uridine kinase in liver and lung (Fig. 1), small intestine (Fig. 2)

Fig. 1. Developmental formation of uridine kinase in rat liver and lung

Uridine kinase activities in the soluble fractions of liver (●) (a) and lung (▲) (b) and in the Triton-treated pellets (○, liver; △, lung) are shown as munits/g of tissue. Points are means of values from three or more litters or three to seven individual animals (bar = 1 s.d.). Where no s.d. is given, results from two animals were averaged. The assay procedure is described in the Experimental section.

and pancreas (Fig. 3) show considerable changes in enzyme amounts in the course of maturation. Soluble uridine kinase in liver underwent a profound decrease in activity at the end of gestation and rose precipitously after birth to reach a peak, 3 times the adult value, about 3 days after birth (Fig. 1a). After day 9 soluble hepatic uridine kinase was approximately at adult values, with only a small rise in

Fig. 2. Maturational changes in uridine kinase in small intestine

Soluble (●) and particle-associated (○) uridine kinase activities were determined in intestine (two pools/litter) of six foetal litters, in total small intestines from three to eight individual rats younger than 5 days and in the upper half of small intestines from three to eight older animals. Only the jejunum was used in adult rats. Points are means of values (bar = 1 s.d.). The assays are described in the Experimental section.

activity immediately preceding the onset of the emergence of hepatic enzymes of the 'late suckling cluster'. In foetal and newborn rats 7–10% of the total hepatic activity was associated with particles. This particulate activity was no longer discernible after day 16. The uridine kinase activity of the lung underwent two short periods of rise: the first of these (1 day after birth) was preceded by a substantial increase in particle-bound uridine kinase (more than 20% of the total activity immediately after birth) (Fig. 1a). In the kidney (results not shown) the total uridine kinase activity also rose transiently but significantly (to 180% of the adult activity) in the first neonatal week. Brain had its highest soluble uridine kinase activity late in gestation (180% of the adult) and high particulate activity throughout the suckling period (150% of adult). The overall developmental changes in the brain were relatively small, however, and the results are not shown. At no time during maturation did liver, lung, kidney or brain uridine kinase activities sink below those of the adult homologous tissue. The developmental pattern in the small intestine differed from those in the other four tissues (Fig. 2): although foetal enzyme activities were half those of the adult jejunum, they were decreased to approx. 15% of the adult values immediately after birth and remained low until about day 14. The subsequent upsurge coincides with the resumption of the active functioning of the pituitary–adrenal–thyroid axes (Levine & Mullins, 1966) and with the onset of a supplementation of the rat pups' diet with their dam's solid food.

Table 3 illustrates that extrauterization, even at a premature age, resulted in the rapid increases in pulmonary and decreases in intestinal uridine kinase. Delivery, whether early or at the normal time, was followed within 2 h by a decrease in the intestinal

Table 3. Effect of premature delivery on uridine kinase activity in liver, lung and small intestine

In Expts. 1 and 2 fetuses from time-mated litters were delivered 48 h before the expected normal parturition time. Half the litters were killed immediately (and the tissues pooled), and the remaining pups were kept wrapped in 0.9% NaCl-soaked gauze in a humidified incubator at 37°C. In each experiment only two of the incubated pups survived for 4 h. In Expt. 3 pups born naturally on day 22 from one litter were removed and killed immediately after birth ($t = 0$) or kept, as described above, for 2 h. Uridine kinase activities were measured in tissue pools.

	Premature delivery			Normal birth	
	$t = 0$	$t = 4$ h		$t = 0$	$t = 2$ h
Liver					
Expt. 1	57.4 ± 3.6 (2 pools)	53.8	Expt. 3	53.2	59.6
Expt. 2	52.8 ± 1.8 (2 pools)	83.0			
Lung					
Expt. 1	75.3	109.0	Expt. 3	88.3	88.7
Expt. 2	40.5	113.0			
Small intestine					
Expt. 1	85.4	32.7	Expt. 3	88.2	58.9
Expt. 2	45.4	—			

enzyme; the postnatal rise in uridine kinase activity of the lung required more than 2 h to become apparent, whereas the neonatal increase of the enzyme in liver was uncertain even 4 h after premature delivery and had not begun 2 h after normal delivery.

Uridine kinase activity in the pancreas was almost undetectably low until the end of the third week

Fig. 3. Normal and altered developmental formation of uridine kinase in rat pancreas

Uridine kinase in two to three pancreas pools (two to four rats per pool) were determined during normal development (●), 18 h after an intraperitoneal injection of 2.5 mg of cortisol/100 g body wt. (○), after 18 h of starvation (▲) and after 18 h of starvation of cortisol-injected pups (△). Points are averaged results from two pools or means from three pools (bar = 1 s.d.).

(Fig. 3). The post-weaning rise could be evoked prematurely by overnight starvation of rats between 13 and 15 days old; even greater enhancement of activities occurred when cortisol-injected rats were deprived of food overnight, but cortisol alone had little (at 13 days) or no effect (at 15 days) on pancreatic uridine kinase activities.

The effects of starvation or cortisol on the enzyme activities during development in the liver and small intestine differed substantially (Table 4). In livers of 9-day-old rats either treatment increased the uridine kinase concentrations only slightly (15–35%; significance shown in legend to Table 4); in 12-day-old rats neither treatment had any effect on the hepatic enzyme concentrations; at both ages overnight starvation led to a loss of approx. 30% of the liver weights. Small intestine of 9-day-old cortisol-treated rats had 560% and starved ones 650% of the activities of control littermates (with 4 and 28% losses in tissue weights respectively). By the age of 12 days, when normal intestinal uridine kinase was about to begin its late-suckling rise (Fig. 2), cortisol merely doubled the low control activities, whereas starvation increased them to over 1900%. At 14–16 days, with the normal enzyme rise well under way, cortisol no longer had any effect on the enzyme in the small intestine, whereas suckling animals, starved overnight, continued to accumulate 5 times the uridine kinase activity of controls. At no age were the effects of cortisol and starvation additive in the small intestine, nor was there a preferential accumulation of particulate uridine kinase. The enzyme in adult liver or jejunum did not increase in response to either cortisol or overnight starvation.

Discussion

Whereas previous publications (e.g. Krystal & Webb, 1971; Greenberg *et al.*, 1977) have con-

Table 4. Effects of starvation or cortisol injection on uridine kinase activities in small intestine and liver. Littermates were untreated, injected with 2.5 mg of cortisol acetate/100 g body wt. or starved. They were killed 18 h after onset of the treatment. Results from one to three litters were averaged for the mean values ($\pm$ s.d.). The significance of the results (*P* value) was calculated by Student's *t* test: **P* < 0.005; †*P* < 0.001.

Tissue	Age (days)	Rat treatment ...	Uridine kinase activity (munits/g)		
			None (control)	Cortisol injection	Starvation overnight
Small intestine	9		7.7 $\pm$ 4.8	43.5* $\pm$ 9.6	49.9† $\pm$ 3.5
	12		2.2 $\pm$ 2.9	5.1 $\pm$ 8.0	42.4† $\pm$ 13.7
	14–16		12.6 $\pm$ 5.9	12.0 $\pm$ 10.3	62.4† $\pm$ 9.6
	60–90		121.0 $\pm$ 24.0	104.0 $\pm$ 19.0	101.0 $\pm$ 9.0
Liver	9		49.1 $\pm$ 2.3	66.6† $\pm$ 0.4	57.1 $\pm$ 4.3
	12		56.6 $\pm$ 4.9	61.9 $\pm$ 9.2	54.6 $\pm$ 0.1
	60–90		26.4 $\pm$ 5.5	27.4 $\pm$ 1.6	28.1 $\pm$ 7.6

centrated on the delineation of the properties of uridine kinase in partially purified preparations, the present report has focused on comparisons of the quantitative distribution of the enzyme in crude rat tissues in the course of normal maturation and under the impact of potential regulators of enzyme activities *in vivo*.

The prevalence of uridine kinase in all adult, neoplastic and most foetal rat tissues contrasts with the distribution of aspartate carbamoyltransferase (EC 2.1.3.2), involved in synthesis of pyrimidines *de novo*, and with that of thymidine kinase, part of the salvage pathway for DNA synthesis; both of the latter enzymes are highly concentrated in foetal and neoplastic tissues (Machovich & Greengard, 1972; Herzfeld & Knox, 1972) and at almost undetectably low activities in non-proliferating adult organs (Herzfeld & Knox, 1972; Machovich & Greengard, 1972). The relative ease with which uridine kinase is inhibited *in vitro* by cytidine, but not by thymidine or deoxyfluorouridine, supports the hypothesis that this enzyme catalyses the phosphorylation of cytidine also and that it may be the catalytic step that is sensitive to chemotherapeutic agents such as 5-azacytidine (Greenberg *et al.*, 1977). The independence of the accumulation of uridine kinase from the growth rate of neoplasms in the rat further indicates that the salvage pathway for RNA synthesis cannot account for the synthesis of all RNA in malignant tissues.

Peaks in the normal developmental formation of uridine kinase in liver precede the emergence of enzyme clusters with heterogeneous functions during critical periods of hepatic maturation (Greengard, 1971). The transient normal rise in hepatic uridine kinase around day 12 is concurrent with the onset of the upsurges of the enzymes of the 'late suckling cluster' [e.g. ornithine aminotransferase (EC 2.6.1.13) (Herzfeld & Greengard, 1969), alanine aminotransferase (EC 2.6.1.12) (Herzfeld *et al.*, 1976), glucokinase (EC 2.7.1.12) (Jamdar & Greengard, 1970) etc.]. In the small intestine uridine kinase has a developmental pattern resembling that of alanine aminotransferase (A. Herzfeld & S. M. Raper, unpublished work) and pyrroline-5-carboxylate reductase (Herzfeld & Raper, 1976). Growth of small intestine is most rapid immediately after birth and again around the time of weaning (Widdowson *et al.*, 1976). Both of these periods coincide with high uridine kinase activities (Herbst *et al.*, 1970). The physiological significance of the intervening trough in the activities of these three enzymes during the suckling period is not known.

The high activities of uridine kinase in most foetal tissues, its absence from foetal pancreas, its postnatal rises in the liver to more than double the adult concentrations and its substantial but transitory decrease in the small intestine during the suckling

period indicate that several physiological factors must regulate its emergence and its maintenance. Uridine kinase is not unique in that its premature developmental formation can be elicited in only some organs by cortisol. In the intestine cortisol may be one of the natural triggers for the accumulation of uridine kinase, since both the high concentrations of cortisone during late gestation and the resumption of the functioning of the pituitary-adrenal axis in the late suckling period (Levine & Mullins, 1966) were accompanied by high enzyme activities; high activities were evoked by a single injection of cortisol during the ebb period of the pituitary-adrenal interaction. The response of intestinal uridine kinase to a single injection of cortisol was more rapid than that usually seen for other cortisol-induced intestinal enzymes (Koldovsky & Palmieri, 1971; Koldovsky & Herbst, 1973; Moog *et al.*, 1971, 1973; Lafont & Pilon, 1975; Yeh & Moog, 1975). Several-fold increases in intestinal uridine kinase followed 18 h of starvation; this trigger for increased enzyme accumulation extended to ages when cortisol was no longer a productive stimulus for raising the enzyme activity. Such an effect suggests that an additional, as yet unknown, stimulus regulates the accumulation of uridine kinase in rat intestine during the latter part of the suckling period. When cortisol was injected into starving 7–11-day-old rats, the enzyme accretion in the small intestine was not synergistic, indicating independent mechanisms of action. In the liver the highest activities occurred during the period of lowest pituitary-adrenal activity; an injection of cortisol did raise hepatic uridine kinase activity by 35% in 9-day-old rats, but this increase was slight when compared with the normal activities during the peak period of enzyme accumulation (days 3–5). In the liver the periods of maximal activities of uridine kinase coincide with the times of highest activity for several amino acid-metabolizing enzymes, e.g. aspartate aminotransferase (EC 2.6.1.1) (Herzfeld & Greengard, 1971), glutamate dehydrogenase (EC 1.4.1.2) (Herzfeld *et al.*, 1973) and pyrroline-5-carboxylate reductase (Herzfeld & Raper, 1976); hepatic activities of these enzymes were not changed by cortisol in suckling rats. Thus the accumulation and decline of uridine kinase activity in the liver, small intestine and pancreas are not regulated by the same stimuli; cortisol stimulated the enzyme only briefly in the three tissues, whereas the effectiveness of short starvation in raising the enzyme activities in the small intestine and pancreas (but never in the liver) persisted over a much longer time. This suggests that the enzyme accumulation by starvation is not mediated by stress (or glucocorticoid secretion). The additive stimulation of pancreatic uridine kinase accumulation by cortisol and starvation further supports by hypothesis that the two stimuli operate by different physiological mechanisms.

This study was supported by U.S. Public Health Service Grants HD 04532 from the National Institute of Child Health and Human Development, DHEW, CA 19232 and CA 22065 from the National Cancer Institute, DHEW, and Biomedical Research Support Grant RR 05591 from the Division of Research Resources, DHEW. This is article no. 635 from the Cancer Research Institute of the New England Deaconess Hospital.

References

- Canellakis, E. S., Jaffe, J. J., Mantsavinos, R. & Krakow, J. S. (1959) *J. Biol. Chem.* **234**, 2096–2099
- Greenberg, N., Schumm, D. E., Hurtubise, P. E. & Webb, T. E. (1977) *Cancer Res.* **37**, 1028–1034
- Greengard, O. (1971) *Essays Biochem.* **7**, 159–205
- Herbst, J. J., Fortin-Magana, R. & Sunshine, P. (1970) *Gastroenterology* **59**, 240–246
- Herzfeld, A. & Greengard, O. (1969) *J. Biol. Chem.* **244**, 4894–4898
- Herzfeld, A. & Greengard, O. (1971) *Biochim. Biophys. Acta* **237**, 88–98
- Herzfeld, A. & Greengard, O. (1972) *Cancer Res.* **32**, 1826–1832
- Herzfeld, A. & Greengard, O. (1977) *Cancer Res.* **37**, 231–238
- Herzfeld, A. & Knox, W. E. (1972) *Cancer Res.* **32**, 1842–1847
- Herzfeld, A. & Raper, S. M. (1976) *Biochim. Biophys. Acta* **428**, 600–610
- Herzfeld, A., Federman, M. & Greengard, O. (1973) *J. Cell Biol.* **57**, 475–483
- Herzfeld, A., Rosenoer, V. M. & Raper, S. M. (1976) *Pediatr. Res.* **10**, 960–964
- Herzfeld, A., Greengard, O. & Warren, S. (1978) *J. Natl. Cancer Inst.* **60**, 825–828
- Jamdar, S. C. & Greengard, O. (1970) *J. Biol. Chem.* **245**, 2779–2783
- Keefer, R. C., Morris, H. P. & Webb, T. E. (1974) *Cancer Res.* **34**, 2260–2265
- Koldovsky, O. & Herbst, J. J. (1973) *Gastroenterology* **64**, 1142–1149
- Koldovsky, O. & Palmieri, M. (1971) *Biochem. J.* **125**, 697–701
- Krystal, G. & Webb, T. E. (1971) *Biochem. J.* **124**, 943–947
- Lafont, J. & Pilon, R. (1975) *Biochim. Biophys. Acta* **392**, 288–298
- Levine, S. & Mullins, R. F., Jr. (1966) *Science* **152**, 1585–1592
- Machovich, R. & Greengard, O. (1972) *Biochim. Biophys. Acta* **286**, 375–381
- Moog, F., Birkenmeier, E. H. & Glazier, H. S. (1971) *Dev. Biol.* **25**, 398–419
- Moog, F., Denes, A. E. & Powell, P. M. (1973) *Dev. Biol.* **35**, 143–159
- Reddy, J. K. & Rao, M. S. (1977) *Science* **198**, 78–80
- Reichard, P. & Sköld, O. (1958) *Biochim. Biophys. Acta* **28**, 376–385
- Sköld, O. (1960a) *Biochim. Biophys. Acta* **44**, 1–12
- Sköld, O. (1960b) *J. Biol. Chem.* **235**, 3273–3279
- Widdowson, E. M., Colombo, V. E. & Artavanis, C. A. (1976) *Biol. Neonate* **28**, 272–281
- Yeh, K.-Y. & Moog, F. (1975) *Dev. Biol.* **47**, 173–184