References and Notes

1. W. E. Nance, *Medicine* **38**, 403 (1959); P. J. Waardenburg, *Acta Genet. Stat. Med.* **7**, 10 (1957); M. G. Bulmer, *The Biology of Twinning in Man* (Clarendon, Oxford, 1970).
2. W. E. Nance, *Prog. Med. Genet.* **3**, 73 (1979); K. Benirschke and C. K. Kim, *N. Engl. J. Med.* **288**, 1276 (1973); P. I. Terasaki, D. Gjertson, D. Bernoco, S. Perdue, M. R. Mickey, J. Bond, *ibid.* **299**, 590 (1978); J. B. Scrimgeour and T. G. Baker, *J. Reprod. Fertil.* **36**, 73 (1974); S. A. Rhine and W. E. Nance, *Acta Genet. Med. Gemellol.* **25**, 66 (1976); C. Danforth, *J. Hered.* **7**, 198 (1916); W. A. Mijsberg, *Acta Genet. Stat. Med.* **7**, 42 (1957); S. Rosin, *Arch. Julius Klaus Stift. Vererbungsforsch. Sozialanthropol. Rassenhyg.* **21**, 6 (1948).
3. The acardiac twin was 12 cm long and weighed 322 g. Except for a patch of hair, its cranial end was amorphous and edematous. There were a large omphalocele, absent upper and malformed lower limbs, and total absence of heart, lungs, liver, and kidneys. An ectopic urogenital anlage had failed to differentiate sufficiently for histologic sex determination. C. Kaplan, K. Benirschke, *Acta Genet. Med. Gemellol.* **28**, 51 (1979); K. Benirschke, V. Des Roches Harper, *Teratology* **15**, 311 (1977); J. S. Deacon, G. A. Machin, J. M. E. Martin, S. Nicholson, D. C. Nwankwo, R. Wintemute, *Am. J. Med. Genet.* **5**, 85 (1980).
4. All monochorionic twins have been considered monozygotic, the type of placentation indicating the time at which the twinning process occurred. Thus, dichorionic diplacental monozygotic twins have been presumed to arise from an early separation of the blastomeres, whereas monochorionic twins have been presumed to arise after implantation as a result of duplication of the inner cell mass. Although monochorionic, the present twins are clearly not monozygotic, and despite the placentation, the twinning event must have occurred at or before conception.
5. P. S. Moorehead, P. C. Nowell, W. J. Mellman, D. M. Battips, D. A. Hungerford, *Exp. Cell Res.* **20**, 613 (1960).
6. M. Seabright, *Lancet* **1971-II**, 971 (1971).
7. The karyotype was 70,XXX+15 in 22 of 31 cells (71 percent) from the skin biopsy and in 7 of 28 cells (25 percent) from the intestinal biopsy. Random chromosome loss accounted for metaphase spreads with fewer than 70 chromosomes. The parental origin of the fourth chromosome 15 could not be identified because of a lack of informative QFQ, C, or NOR heteromorphisms.
8. Data on ten acardiac fetuses include four cases in which the acardiac twin was aneuploid (trisomic or monosomic for a single chromosome) and the other twin was chromosomally normal (*3*). Identical twins differing in chromosome complement for a single chromosome can readily be explained by postzygotic nondisjunctional events. Because several of the acardiac twins had normal chromosomes, aneuploidy cannot be the only etiology. K. Benirschke postulated that acardia might arise from fertilization of a polar body, but was unable to confirm his hypothesis, by demonstration of sex discordance, in a sex chromatin survey of six preserved pathologic specimens [*Obstet. Gynecol.* **14**, 72 (1959)]. Many different primary abnormalities of early embryogenesis could result in acardia. If the defect occurs in a twin pregnancy, a reversal of blood flow through placental vascular anastomoses may sustain the development of the defective embryo, but also induce the morphogenetic defects associated with acardia. Thus all acardiacs, regardless of their karyotypes, would have a similar phenotype, varying with the degree of perfusion by the normal twin. In the present case, the cytoplasmic deficiency of the polar body, the presence of triploidy or the tetrasomy 15 may have contributed to the primary defect in embryogenesis.
9. The morphology and staining characteristics of the stalks, satellites, and centromeres of specific human chromosomes are heteromorphic. The heteromorphisms are inherited as Mendelian traits and are characteristic for a particular chromosome in an individual. P. A. Jacobs, *Prog. Med. Genet.* **2**, 251 (1977); V. D. Markovic, R. G. Worton, J. M. Berg, *Hum. Genet.* **41**, 181 (1978).
10. S. E. Bloom and C. Goodpasture, *Hum. Genet.* **34**, 199 (1976); H. J. Evans, R. A. Buckland, M. L. Pardue, *Chromosoma* **48**, 405 (1974); C. Goodpasture, S. E. Bloom, T. C. Hsu, F. E. Arrighi, *Am. J. Hum. Genet.* **28**, 559 (1976). Since NOR heteromorphisms may be functional rather than structural in origin, their expression might be altered by the triploid state. Reported observations in mosaic trisomy [R. D. Wegner, P. Aldenhoff, K. Sperling, *Hum. Genet.* **55**, 227 (1980)] and Robertsonian translocation [H. Zankl and K. D. Zang, *ibid.* **52**, 119 (1979)] have led to conflicting interpretations about the heritability of NOR heteromorphisms in the presence of numerical alterations of the rDNA (ribosomal RNA-encoding gene) regions. In the acardius, all three homologs for chromosomes 14 and 21 were NOR-positive, indicating that trisomic expression of these markers is possible in the triploid state.
11. D. B. Amos, H. Bashir, W. Boyle, M. MacQueen, A. Thlikainen, *Transplantation* **7**, 220 (1969).
12. T. Mohanakumar, R. S. Metzgar, D. S. Miller, *J. Natl. Cancer Inst.* **52**, 1435 (1974).
13. P. A. Jacobs, R. R. Angell, I. M. Buchanan, T. J. Hassold, A. M. Matsuyama, B. Manuel [*Ann. Hum. Genet.* **42**, 49 (1978)] estimated that triploidy among spontaneous abortuses was dispermic in 66 percent, diplospermic in 24 percent, and digynic from a diploid egg formed by failure of first meiotic division in 10 percent.
14. K. Funaki and K. Mikamo, *Cytogenet. Cell Genet.* **28**, 159 (1980); J. F. Kennedy and R. P. Donahue, *Lancet* **1969-I**, 754 (1969).
15. W. J. Hamilton and H. W. Mossman, *Human Embryology* (Williams and Wilkins, Baltimore, 1972).
16. G. B. Wislocki, *Am. J. Anat.* **64**, 445 (1930); K. Bernischke, *Curr. Top. Pathol.* **51**, 1 (1970).
17. R. S. Thompson and L. Zamboni, *Am. J. Anat.* **142**, 233 (1975).
18. W. W. Zuelzer, K. M. Beattie, L. E. Reisman, *Am. J. Hum. Genet.* **16**, 38 (1964).
19. B. Mintz, *J. Exp. Zool.* **157**, 273 (1964); *Proc. Natl. Acad. Sci. U.S.A.* **58**, 344 (1967).

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Novel Single-Pass Exchange of Circulating Uridine in Rat Liver

Abstract. *Evidence is presented that the liver effects an essentially complete degradation of plasma uridine in a single pass and replaces it largely from hepatic pools of acid-soluble uridine nucleotides. The concentration of uridine in the hepatic vein of the rat was essentially the same as that in the arterial circulation and portal vein. However, the isolated perfused rat liver degraded more than 90 percent of infused [5-^{3}H]uridine in a single passage. Similar results were found in vivo when tracer amounts of [^{3}H]uridine and [^{14}C]uridine were infused into the portal vein of an intact rat. Furthermore, less than 2 percent of the infused uridine entered the acid-soluble nucleotide pools of the liver after 30 minutes of infusion. Intraperitoneal injection of [^{3}H]orotate allowed selective labeling of liver (and kidney) pyrimidines. After 3 hours, the specific activity of uridine in the hepatic vein was more than three times that in the arterial circulation. This unusual exchange, which is not saturated even at uridine concentrations as high as 50 μM, contributes to the rapid turnover of plasma uridine and explains its inefficient utilization in peripheral tissues.*

The rapid turnover of plasma uridine suggests that this circulating nucleoside may have a role in the metabolism of pyrimidines by various tissues (*1*, *2*). Defining the location and quantitative significance of this process has recently become possible by the use of new techniques in high-performance liquid chromatography. Studies with phosphonacetyl aspartic acid (PALA) and pyrazofurin, potent inhibitors of the synthesis de novo of pyrimidines, indicate that selective depletion of ribonucleotide pools occurs in some tumors, but that the pools are essentially unaffected in normal tissues and insensitive tumors (*3*, *4*). These observations suggest that circulating pools of pyrimidines could rescue depleted tissues. The source of these pools has not yet been clearly established, but preliminary clinical studies showed that they are relatively unaffected by high doses of PALA (*5*). The liver provides preformed purines to other tissues through the vascular system (*6*), and a similar process has been suggested for pyrimidines (*7*). We therefore undertook a quantitative analysis of the uptake and metabolism of uridine by the liver.

A very rapid turnover [half-time ($t_{1/2}$) ~ 3 minutes] of plasma uridine (1 to 2 μ*M*) has been observed in rats (*2*) and dogs (*8*). In rat plasma, cytidine concentrations are somewhat higher (3 to 5 μ*M*), and the turnover is less rapid ($t_{1/2}$ ~ 25 minutes). Since the liver is the primary location of enzymes responsible for uridine catabolism, the fate of [5-^{3}H]uridine perfused through an isolated rat liver was examined. During a 30-minute period, the radioactivity associated with uridine in the circulating medium decreased with an apparent $t_{1/2}$ of 4.8 minutes; total clearance in a single pass in this apparatus would give a $t_{1/2}$ of 4.9 minutes (Table 1). Thus the liver is extremely efficient at extracting and degrading uridine. Yet in fed, intact rats the concentration of uridine in plasma leaving the liver in the hepatic vein (mean ± standard deviation = 1.32 ± 0.45 μ*M*, *N* = 24) was, if anything, slightly higher than the concentration in portal (1.03 ± 0.3 μ*M*, *N* = 11) or arterial (1.06 ± 0.2 μ*M*, *N* = 21) blood.

To obtain data on the turnover of

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Table 1. Clearance of uridine by the liver in vitro and in vivo. Isolated rat livers were perfused at 20 ml/min from a bath containing 144 ml of Fisher's medium supplemented with 5 percent albumin and adjusted to a 25 percent hematocrit with rat erythrocytes. Earlier studies have shown that uridine equilibrates between plasma and erythrocytes, but that it is not metabolized in these cells during this period, nor do the cells contribute significant amounts of uridine (*4*). Samples from the bath were centrifuged to remove cells, and after perchloric acid precipitation of proteins, [5-^{3}H]uridine was determined by preliminary separation on a borate affinity column (Affigel 601) (*12*) and chromatography on a HAX-4 anion-exchange column (*13*). First-order clearance was observed when the perfusing solution contained 1 to 10 μM [5-^{3}H]uridine. Infusions were made in vivo with a 26-gauge needle into the portal vein at 1.5 μCi of [5-^{3}H]uridine (26 Ci/mmole) or 0.6 μCi of [2-^{14}C]uridine (53 mCi/mmole) per minute. Clearance is based on an estimated portal blood flow of 15 ml/min in the 250-g rats. Livers were perfused in situ with different concentrations of [5-^{3}H]uridine at 10 ml/min, with the medium that was used for isolated perfusions, for up to 45 minutes with a single-pass technique. Clearance was determined as above.

Substance	Clearance per pass (%)
Isolated perfused liver	
[5-^{3}H]Uridine, 1 μM	95
[5-^{3}H]Uridine, 10 μM	95
Infusion in vivo	
[5-^{3}H]Uridine, 1 μM	90
[2-^{14}C]Uridine, 1 μM	85
Single-pass perfusion in situ	
[5-^{3}H]Uridine, 1 μM	90
[5-^{3}H]Uridine, 10 μM	90
[5-^{3}H]Uridine, 50 μM	73

uridine under physiological conditions, we infused [5-^{3}H]uridine in tracer amounts into the portal vein without inhibiting flow. In this system, more than 90 percent clearance was observed (Table 1). This was not a ^{3}H-exchange process since [2-^{14}C]uridine gave similar results. Under these conditions less than 2 percent of the [^{3}H]uridine entered the pool of acid-soluble pyrimidine nucleotides in the liver. These results suggest that almost none of the uridine in plasma entering the liver leaves through the hepatic vein and that uridine in the hepatic vein is probably derived from de novo synthesis.

In the rat, orotate, the penultimate precursor of uridine nucleotides on the de novo pathway, is selectively concentrated by the liver and kidney (*9, 10*). Thus it was possible to label the acid-soluble pyrimidine ribonucleotide pool of the liver with [5-^{3}H]orotate and to observe the contribution of this pool to uridine leaving the liver (Table 2). The specific activity of plasma from the hepatic vein was more than three times that in the arterial circulation and approaches the specific activity of the acid-soluble pools in the liver. The difference might reflect heterogeneity of specific activity within the acid-soluble pools or a substantial contribution of uridine from turnover of RNA of lower specific activity through 2′,3′-uridylate to uridine.

To determine the limits of the chemostatic capacity of the liver, a single-pass perfusion system in situ was established. The portal vein and the bile duct were cannulated; the hepatic artery and the inferior vena cava were ligated in the abdominal cavity. The perfusion medium was collected from the vena cava in the thoracic cavity. The flow rate was 10 ml/min, and bile flow was monitored throughout the experiment to assure sustained hepatic function. When the concentration of [^{3}H]uridine (5 μCi/μmole) was 1 μM, the radioactivity associated with uridine in the exiting perfusate was again less than 10 percent of that which had entered (Table 1). Similar results were obtained at uridine concentrations of 5 and 10 μM. Even at concentrations as high as 50 μM, only 27 percent of the infused radioactivity exited as unchanged uridine. The contribution of uridine derived from the degradation of nucleic acids in rat food to portal blood would approximate 20 μM if there were no degradation of the uridine in the intestinal lumen or utilization by the visceral organs before passage through the liver. However, in mice fed [^{14}C]pyrimidine-labeled RNA, less than 3 percent of the total ingested pyrimidines was utilized for nucleotide synthesis (*11*). Apparently degradative reactions compensate for the contribution of the diet to portal plasma uridine in the rat, since the concentration of uridine in portal blood is approximately equal to that in arterial blood. Thus the capability of the liver to degrade uridine is not saturated at concentrations many times greater than physiological levels in fed rats.

These data suggest a unique and simple (though not very economical) mechanism for regulation of uridine concentrations in the circulation in which no sensor-effector communication is necessary. The liver appears to degrade essentially all incoming uridine contained in the portal drainage derived from intestinal absorption and peritoneal organs. Hepatic pools of acid-soluble nucleotides, formed almost completely by de novo synthesis, are responsible for the uridine concentrations in plasma leaving the liver through the hepatic vein. The controls on this process and changes in the flux under conditions of increased peripheral requirements and antimetabolite inhibition remain to be established.

Table 2. Specific activity of acid-soluble uridine nucleotides, RNA, and plasma uridine after injection of [^{3}H]orotate. [5-^{3}H]Orotate (19 Ci/mmole, 500 μCi/kg) was injected intraperitoneally into male Sprague-Dawley rats (250 to 350 g). After 3 hours, the animals were anesthetized with pentobarbital (50 mg/kg), and blood and organ samples were taken. For the determination of the specific activity of acid-soluble uridine nucleotides, organs were frozen quickly in liquid nitrogen and homogenized in 5 volumes of 1 M perchloric acid. The acid-soluble supernatant was hydrolyzed at 100°C for 14 minutes, neutralized with KOH, and then chromatographed on a SAX-10 anion-exchange column (*4*). Radioactivity in the RNA was determined after extraction of the acid-soluble fraction with 1 M NaOH. Blood samples were processed as described in Table 1 to determine the specific activity of plasma uridine.

Source	Specific activity (μCi/μmole)		
	Acid-soluble uridine nucleotides	RNA	Plasma uridine
Liver	2.4	0.2	
Kidney	1.8	0.18	
Spleen	0.06	0.002	
Intestine	0.04	0.004	
Lung	0.03	0.003	
Hepatic vein			1.42
Portal vein			0.20
Aorta			0.43

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References and Notes

1. H. Dahnke and K. Mosebach, *Hoppe-Seyler's Z. Physiol. Chem.* **356**, 1565 (1975).
2. J. D. Moyer, J. T. Oliver, R. E. Handschumacher, *Cancer Res.* **41**, 3010 (1981).
3. D. E. Dix, C. P. Lehman, A. Jakubowski, J. D. Moyer, R. E. Handschumacher, *ibid.* **39**, 4485 (1979).
4. J. D. Moyer and R. E. Handschumacher, *ibid.*, p. 3089.
5. J. M. Karle, L. W. Anderson, C. Erlichman, R. L. Cysyk, *ibid.* **40**, 2938 (1980).
6. J. B. Pritchard, N. O'Connor, J. M. Oliver, R. D. Berlin, *Am. J. Physiol.* **229**, 967 (1975).
7. R. L. Levine, N. J. Hoogenraad, N. Kretchmer, *Pediatr. Res.* **8**, 724 (1974).
8. J. Tseng, J. Barelkovski, E. Gurpide, *Am. J. Physiol.* **221**, 869 (1971).
9. M. G. Ord and L. A. Stocken, *Biochem. J.* **132**, 47 (1973).
10. M. A. Lea, J. Bullock, F. L. Khalil, H. P. Morris, *Cancer Res.* **34**, 3414 (1974).
11. T. Sonoda and M. Tatibana, *Biochim. Biophys. Acta* **521**, 55 (1978).
12. C. W. Gehrke, K. C. Kuo, G. E. Davis, R. D. Suits, T. P. Waalkes, E. Borek, *J. Chromatogr.* **150**, 445 (1978).
13. R. P. Singhal and W. E. Cohn, *Anal. Biochem.* **45**, 585 (1972).
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