

# Metabolism of Deoxycytidine, Thymine, and Deoxythymidine in the Hamster<sup>1</sup>

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## SUMMARY

The ability of growing and of mature Syrian hamsters to anabolize (to liver DNA) or catabolize (to <sup>14</sup>CO<sub>2</sub>) graded amounts of [2-<sup>14</sup>C]deoxythymidine (TdR), thymine, or deoxycytidine (CdR) was measured *in vivo*. Of the three precursors, CdR labeled DNA most efficiently and, as expected, incorporation of all three into DNA was greater in younger animals. The catabolism of [2-<sup>14</sup>C]CdR to respired <sup>14</sup>CO<sub>2</sub> was dose dependent and showed no signs whatsoever of saturation, even with the highest dose (>20 μmoles/g liver). In contrast, TdR and thymine were catabolized more slowly and saturation was approached with modest doses. The excretion of CdR in the urine was low and independent of dose, while excretion of TdR and thymine was greater and was dose dependent. Rats tested with an intermediate dose of CdR did not catabolize significant quantities to <sup>14</sup>CO<sub>2</sub>, but did excrete considerably more [<sup>14</sup>C]CdR into the urine than did hamsters. These and other findings suggest that, while the rat and the hamster metabolize thymine (and TdR as well) in a similar fashion, they metabolize CdR quite differently, probably because the hamster has a much higher level of nucleoside aminohydrolase which deaminates CdR and related compounds. Because the human also has a very high level of this enzyme, the hamster appears to be a superior animal model for the study of cytosine-containing compounds intended for human use.

## INTRODUCTION

It is widely recognized that DNA synthesis is accompanied by an increase in TdR<sup>4</sup> kinase activity and that incorporation of TdR into DNA can be used to detect, if not always quantitate, DNA synthesis (6). It is also recognized that in the mammal the catabolism of pyrimidines may have a profound effect on *in vivo* studies of DNA synthesis when radioactive TdR is used as a precursor (6, 34). Indeed,

several attempts to inhibit the rate-limiting step in pyrimidine catabolism (dihydropyrimidine dehydrogenase) have been reported (7, 8, 15). Others (11) have sought to determine the relationship between the TdR anabolic-catabolic balance and tissue growth status (*i.e.*, differentiating *versus* regenerating *versus* neoplastic rat liver).

The metabolism of CdR, in contrast, has received less attention, although CdR can be used to label DNA specifically. This study was undertaken to define the optimal conditions for labeling of hamster liver DNA *in vivo* with TdR and CdR. It is shown that CdR, although much more efficient than TdR as a precursor of DNA, is also catabolized much more rapidly than is TdR or thymine. Further, TdR and thymine are excreted in significant amounts via the urine while CdR is not.

## MATERIALS AND METHODS

[2-<sup>14</sup>C]thymine, [2-<sup>14</sup>C]TdR, and [2-<sup>14</sup>C]CdR (New England Nuclear, Boston, Mass.) contained no significant impurities as determined by paper chromatography. The unlabeled compounds (Calbiochem, Los Angeles, Calif.) were of the highest purity. Solutions for injections were prepared by diluting the <sup>14</sup>C-labeled pyrimidines with aqueous solutions of the corresponding unlabeled compounds, Evans blue dye was added at a final concentration of 0.05%, and these were stored frozen until used. All assays for radioactivity were by liquid scintillation counting (16).

Male hamsters (Lakeview Hamster Colony, Newfield, N. J.) were given injections *i.p.* and placed immediately in metabolism chambers for the collection of urine and expired <sup>14</sup>CO<sub>2</sub>. The Evans blue dye was used to detect *s.c.* or *intra*intestinal injections (29). Because of previous experiments by Morse (29) and Potter *et al.* (33), which indicated the importance of circadian rhythms in mammalian nucleic acid metabolism, the hamsters were housed in quarters illuminated daily from 8:00 a.m. until 8:00 p.m. for at least 10 days prior to any experiments. For reduction of possible variation from such rhythms, all experiments were begun at the same time of day (noon) plus or minus 1 hr. The animals were fed Purina laboratory chow and offered water *ad libitum*. For purposes of comparing growing animals with adults the hamsters were used when they had reached 50 g (22 to 26 days old) or 100 g (55 to 65 days old) body weight.

<sup>14</sup>CO<sub>2</sub> was trapped in NaOH that was diluted 1/10 in distilled water and counted in a liquid scintillation spectrometer. When the <sup>14</sup>CO<sub>2</sub> collection was completed all the urine voided up to that time, plus that remaining in the

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<sup>4</sup> The abbreviations used are: TdR, deoxythymidine; CdR, deoxycytidine.

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bladder at sacrifice, was collected and diluted to 100 ml with distilled water, and aliquots were counted. Paper chromatography of urine samples using Fink's (12, 13) System 7 for thymine and System 8 for the nucleosides showed that radioactivity was present exclusively as the injected precursor. The chromatographs were analyzed qualitatively with a Vanguard automatic chromatogram scanner, and the areas containing the radioactivity were identified by comparison with UV-absorbing spots on control chromatograms of corresponding unlabeled pyrimidines. No attempt was made to determine radioactivity of fecal material.

Livers were fractionated essentially as described by Morse (29) and Hurlbert and Potter (21). Aliquots of the DNA, RNA, and acid-soluble fractions were counted. No significant counts were recorded in the RNA fraction. The amount of DNA was assayed by the Dische (9) diphenylamine reaction, with deoxyadenosine used as a standard.

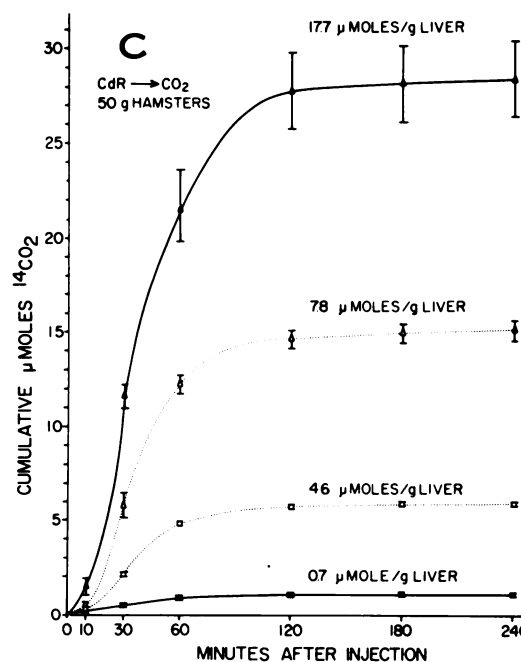
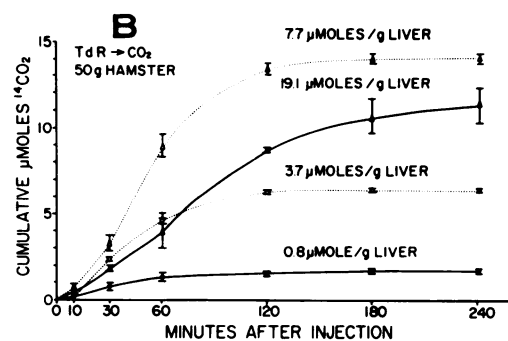
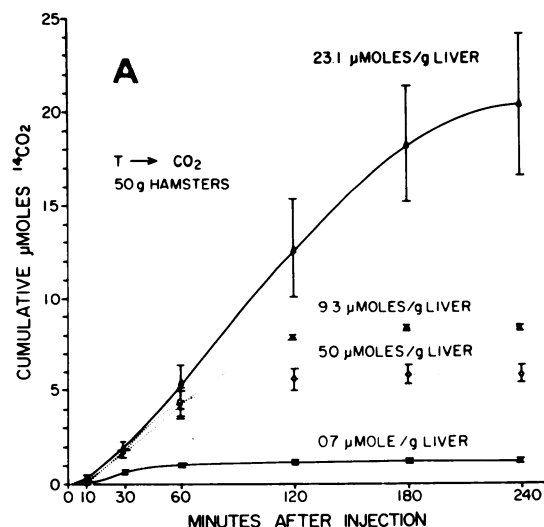
## RESULTS

Respiratory  $^{14}\text{CO}_2$  output following injection of pyrimidines labeled in the 2 position is shown by cumulative graphs which indicate time for completion of catabolism, and by rate plots derived from the cumulative data (29). The latter show the time required to reach maximum output as well as maximum rates.

Animals received 2, 10, 30, or 45  $\mu\text{moles}$  of test compound, but because of the variation in liver weights, doses are expressed as  $\mu\text{moles/g}$  of liver. Cumulative  $^{14}\text{CO}_2$  output data and rate data for TdR, thymine, and CdR in 50-g hamsters may be seen in Charts 1 and 2. Data obtained with 100-g hamsters (not shown) were similar. In general, the time to completion of catabolism is dose dependent, although the catabolism of CdR and TdR appears to go to completion somewhat more rapidly than that of thymine. When the maximum catabolic rates for 50- and 100-g hamsters are plotted as a function of dose (Chart 3), it can be seen that saturation of the catabolic system has nearly been achieved at the highest doses of thymine and possibly of TdR. In sharp contrast to these results, the data for CdR indicate no apparent approach to rate saturation at the highest levels tested. Further inspection of the rate data (Chart 2) shows that the time of maximum catabolic rate is a function of the dose for thymine and TdR but not for CdR. Similar results have been reported for thymine, uracil, and TdR in the rat (29).

Excretion patterns of radioactivity in the urine of 50-g animals receiving either thymine or TdR were similar, the percentage excreted being a function of dose (Charts 4 and 5). In contrast, CdR excretion in the urine was uniformly

Chart 1. *A*, excretion of  $^{14}\text{CO}_2$  from [2- $^{14}\text{C}$ ]thymine by the young male hamster. *Curves*, averages from at least 2 animals. Dosages given i.p. in 1-ml aqueous solutions were 2  $\mu\text{moles}$  (2.5  $\mu\text{Ci}/\mu\text{mole}$ ), 10  $\mu\text{moles}$  (0.5  $\mu\text{Ci}/\mu\text{mole}$ ), 20  $\mu\text{moles}$  (0.25  $\mu\text{Ci}/\mu\text{mole}$ ), and 45  $\mu\text{moles}$  (0.11  $\mu\text{Ci}/\mu\text{mole}$ ). The dose per g of liver was calculated for each. *Bars*, extreme values. *B*, excretion of  $^{14}\text{CO}_2$  from [2- $^{14}\text{C}$ ]TdR by the young male hamster. For details see *A*. *C*, excretion of  $^{14}\text{CO}_2$  from [2- $^{14}\text{C}$ ]CdR by the young male hamster. For details see *A*.



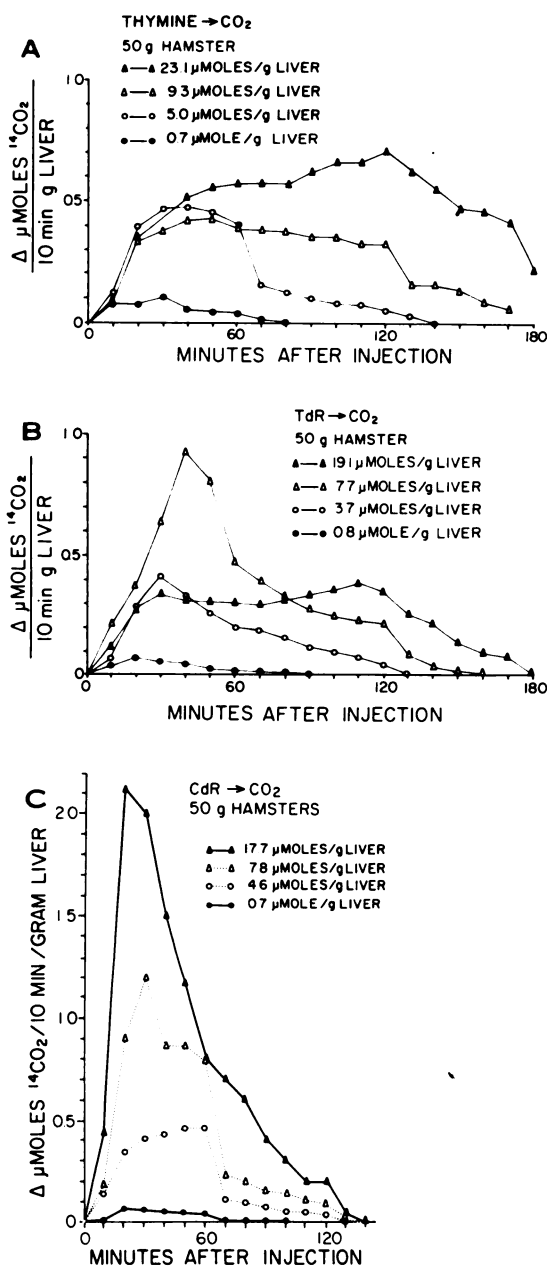


Chart 2. *A*, rate of excretion of  $^{14}\text{CO}_2$  from  $[2\text{-}^{14}\text{C}]$ thymidine by the young male hamster, calculated from data in Chart 1*A*. *B*, rate of excretion of  $^{14}\text{CO}_2$  from  $[2\text{-}^{14}\text{C}]$ TdR by the young male hamster, calculated from data in Chart 1*B*. *C*, rate of excretion of  $^{14}\text{CO}_2$  from  $[2\text{-}^{14}\text{C}]$ CdR by the young male hamster, calculated from data in Chart 1*C*.

low in all cases (Chart 6). The 100-g animals showed similar patterns.

Incorporation of radioactive pyrimidines into liver DNA showed a marked difference when data from 50- and 100-g hamsters were compared on the basis of dose per unit of liver weight. All 3 precursors showed greater incorporation in the young animals (Chart 7). Of the 3, CdR appeared to be the most efficient precursor of DNA pyrimidines and thymine the least efficient.

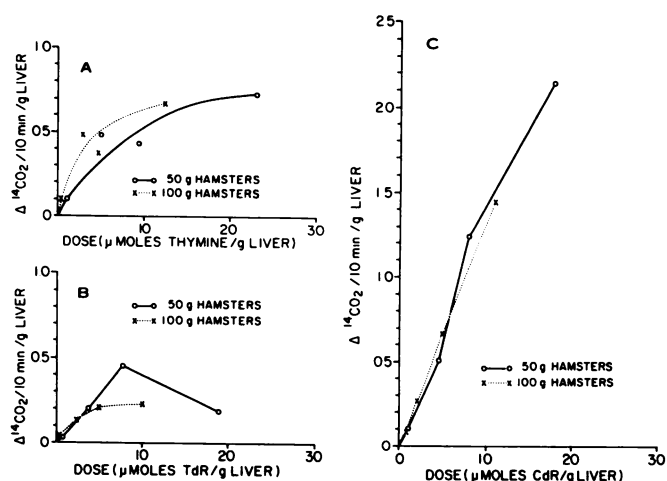


Chart 3. *A*, maximum rate plots for various doses of thymine, constructed using data from Chart 2*A*,  $\Delta\text{ }^{14}\text{CO}_2$ ,  $\Delta\text{ } \mu\text{moles } ^{14}\text{CO}_2$ . *Points*, averages from at least 2 animals. *B*, maximum rate plots for various doses of TdR, constructed using data from Chart 2*B*. Other details in *A*. *C*, maximum rate plots for various doses of CdR, constructed using data from Chart 2*C*. Other details as in *A*.

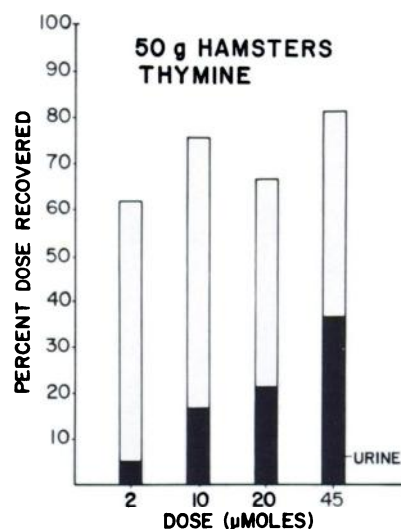


Chart 4. Total recovery of radioactivity from 50-g hamsters 4 hr after administration of  $[2\text{-}^{14}\text{C}]$ thymine. *Shaded area*, percentage of dose recovered as the injected precursor in the urine. *Unshaded area*, percentage recovered as  $^{14}\text{CO}_2$ . *Bars*, average from at least 2 animals. See Chart 1*A* for  $^{14}\text{CO}_2$  data.

In order to facilitate a comparison with similar studies in the rat (29), and because earlier unpublished experiments (J. E. Coward) had suggested that  $[2\text{-}^{14}\text{C}]$ CdR was not metabolized to  $^{14}\text{CO}_2$  in this species, the following experiment was carried out. Two 50-g hamsters and two 100-g male Holtzman rats (The Holtzman Co., Madison, Wis.) were given 10  $\mu\text{moles}$  of  $[2\text{-}^{14}\text{C}]$ CdR as described. The results, shown in Table 1, show that in marked contrast to the hamsters, the rats did not respire significant amounts of  $^{14}\text{CO}_2$ , although substantially more radioactivity was recovered in the urine of the rats.

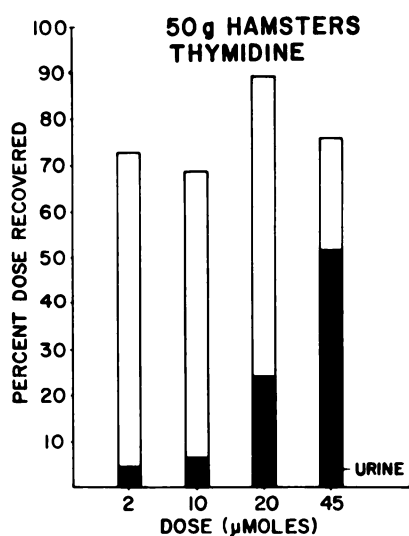


Chart 5. The excretion of  $^{14}\text{CO}_2$  from  $[2-^{14}\text{C}]\text{TdR}$  and as  $[2-^{14}\text{C}]\text{TdR}$  recovered in the urine of young male hamsters. See Chart 1B for  $^{14}\text{CO}_2$  data. For other experimental details see Chart 4.

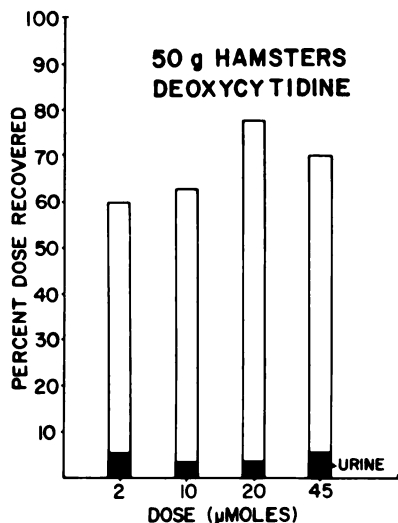


Chart 6. The excretion of  $^{14}\text{CO}_2$  from  $[2-^{14}\text{C}]\text{CdR}$  and as  $[2-^{14}\text{C}]\text{CdR}$  recovered in the urine of young male hamsters. See Chart 1C for  $^{14}\text{CO}_2$  data. For other experimental details see Chart 4.

## DISCUSSION

**Catabolism of Thymine and TdR.** From Chart 2A it may be estimated that the 50-g hamster can catabolize thymine at 4  $\mu\text{moles/hr/g}$  of liver at saturation. Other studies with rats have given similar results, both *in vivo* (29, 35, 36) and *in vitro* (14, 30).

**Age and Dose Dependence of Thymine and TdR Incorporation.** While incorporation of all 3 precursors shows a direct dependence on dose, TdR was a more efficient DNA precursor than thymine in both sizes of animals (Chart 7). Selection of 50- and 100-g hamsters was made in part because it was assumed that the rate of liver DNA synthesis would be greater in the younger animals, permitting the examination of growth status as a variable.

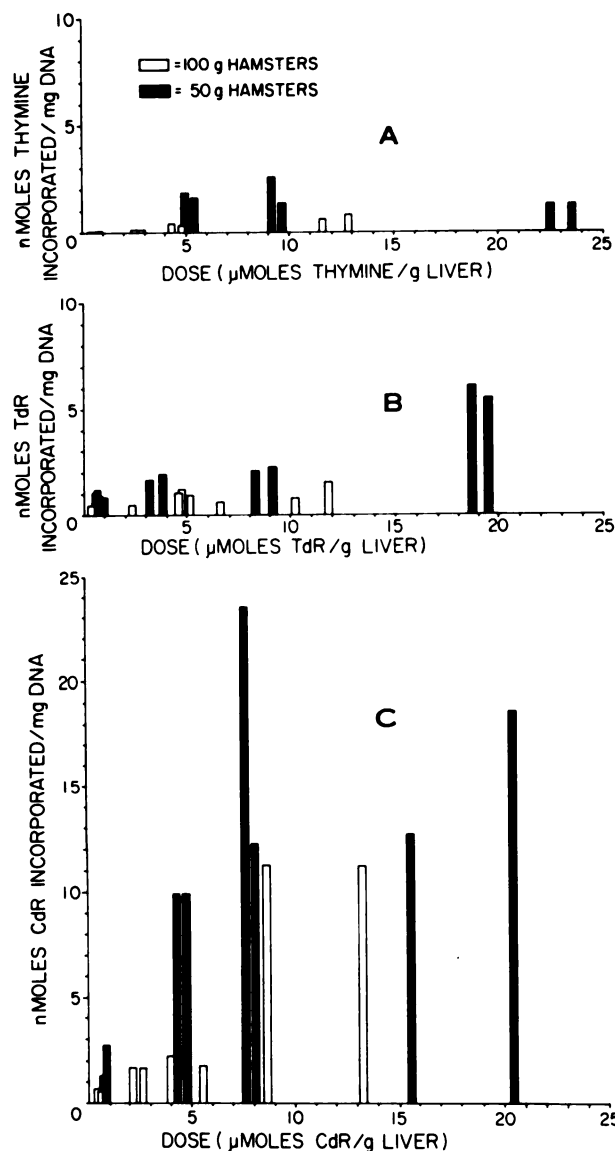


Chart 7. Incorporation of radioactivity into hamster liver DNA from each of the injected precursors. Bars, 1 animal each. A, thymine incorporation; B, TdR incorporation; C, CdR incorporation.

Table 1  
Catabolism of  $[2-^{14}\text{C}]\text{CdR}$  to  $^{14}\text{CO}_2$

	Rat <sup>a</sup> (μmoles)	Hamster <sup>b</sup> (μmoles)
Respiration		
0-30 <sup>c</sup>	0	1.78
30-60	0.15	3.19
60-120	0.19	2.17
120-180	0.18	0.42
180-240	0.19	0.09
Urine	1.22	0.59
Total	1.93	8.24

<sup>a</sup> Average of 2 rats each weighing 100 g.

<sup>b</sup> Average of 2 hamsters each weighing 50 g.

<sup>c</sup> Number of min after injection of 10  $\mu\text{moles}$ .

As expected, incorporation was higher in the younger animals.

**Urinary Excretion of Thymine and TdR.** The output of radioactive thymine and TdR in the urine was also dose related (Charts 4 and 5); with larger doses the peak catabolic rate was delayed. This should have allowed these compounds to persist longer in the posthepatic circulation which would increase their elimination by the kidneys. In contrast, however, trace amounts of [<sup>3</sup>H]TdR given i.v. to humans were catabolized by the liver so rapidly that complete mixing in the plasma (and subsequent availability to the kidney) was never approached (34).

**Catabolism of CdR.** The rapid catabolism of [2-<sup>14</sup>C]CdR to <sup>14</sup>CO<sub>2</sub> probably depends on the conversion of CdR to deoxyuridine by liver and kidney nucleoside aminohydrolase, which is found in unusually high levels in the hamster (4), and the subsequent conversion of deoxyuridine to uracil and dihydrouracil (36). If this is the correct pathway, several conclusions can be drawn for which there is independent evidence.

CdR must cross the cell membrane without being phosphorylated. Several studies of deoxyribonucleoside transport suggest that this can occur (3, 22, 26, 28, 31, 38, 39). Further it may be inferred from the data of Hay *et al.* (19) that, in cultured baby hamster kidney cells that lack CdR kinase (23, 24), 5-fluorodeoxycytidine is converted directly to 5-fluorodeoxyuridine without being phosphorylated.

Substantial catabolism of CdR should not depend on interconversions at the nucleotide level. In the rat, which has all the necessary enzymes (including CdR kinase) for catabolism of CdR via nucleotide interconversions (1, 37) but which has hardly any nucleoside aminohydrolase (4), CdR is not significantly catabolized. Substantial catabolism of CdR also should not occur by way of cytosine. Mammals appear not to metabolize cytosine at all (2, 27). This property explains the usefulness of the cytosine analog 5-fluorocytosine in man, who does not metabolize it, in treating diseases caused by yeasts that do (18, 25, 32). In addition, preliminary experiments (M. T. Dorsett) suggested that crude extracts, as well as partially purified dihydropyrimidine dehydrogenase from hamster liver, showed no activity with cytosine.

**Rate-limiting Steps in Pyrimidine Catabolism.** It might be expected that CdR would be catabolized much faster than TdR and thymine because dihydropyrimidine dehydrogenase, long thought to be the rate-limiting step in mammalian pyrimidine catabolism (5, 13, 14), has a greater activity *in vitro* toward uracil than thymine (10).

This would not, however, explain why CdR labeled DNA much more efficiently than did TdR or thymine (Chart 7); if the conversion to dihydropyrimidines were rate limiting and if uracil (derived from injected CdR) were catabolized faster than thymine (derived from injected TdR), it would be expected that TdR would persist longer and be the better label, which it was not. For this reason we suspect that other differences, such as in the way CdR, TdR, and thymine are transported into the cell (31), may also be involved; but their exact role, if any, remains to be determined.

**Species Differences in CdR Metabolism.** Nucleoside

aminohydrolase levels vary widely with species. In a study of several different mammals, Zícha and Buřič (4, 40) found that the activity of this enzyme was very high in the liver and kidneys of hamster and calves and was even higher in human liver. In marked contrast, it was hardly measurable in the rat and the pig. Our data are consistent with these studies. They also agree with those of Gerber *et al.* (17) who found that [<sup>14</sup>C]CdR was catabolized rapidly by isolated perfused mouse liver, which has substantial nucleoside aminohydrolase, but not by rat liver, which has little if any.

Finally, the species differences in enzyme levels mentioned above led Zícha and Buřič (4, 40) to suggest that the hamster should be a superior experimental animal for the study of cytosine-containing chemotherapeutic, such as arabinosyl cytosine, ultimately intended for human use. Our data support this suggestion by showing that the *in vivo* metabolism of CdR by hamsters is consistent with a high hepatic level of nucleoside aminohydrolase.

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