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KINETICS OF TRANSPORT AND METABOLISM OF 1-β-D-ARABINOFURANOSYLCYTOSINE AND STRUCTURAL ANALOGS BY EVERTED PERFUSED RAT JEJUNUM

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Abstract—Few reports have dealt with the kinetics and metabolism of AraC and analogs by rat intestine. Using everted rat jejunum with continuous perfusion, it was possible to demonstrate that AraC and Cyd cross the intestinal barrier(s) by a carrier mediated process which was saturable and exhibited fairly good fitting of the flux rate by Michaelis–Menten equation. The transport rate of different analogs was not consistent with the pH-partition theory of membrane transport of drugs being rather dependent on the chemical structure of the nucleoside. A free amino group of cytosine increased the rate of transport within the present series of AraC analogs. There was a detectable deaminase as well as esterase activity towards AraC and its analogs in rat jejunum.

1-β-D-Arabinofuranosylcytosine† (AraC) is one of the most important drugs in the treatment of acute leukemia [1–3] among many other synthesized nucleosides analogs of potential antineoplastic action. Clinical and pharmacological studies indicated poor absorption from the gastrointestinal tract [4–5]. Therefore, the methods of AraC administration commonly used in clinical practice are either intravenous bolus injection or continuous intravenous infusion, since AraC therapeutic effect in the treatment of leukemia largely depends on dosage and mode of administration.

Poor absorption of AraC and the consequent low chemotherapeutic activity is usually attributed to the transformation of AraC to AraU whether by intestinal microflora or by intestinal deaminases [4–6]. Therefore Hanze *et al.* [6] prolonged the action of oral AraC by the inclusion of hydrogenated pyrimidine nucleoside as deaminase inhibitor. However, with the exception of the study of Sciorelli *et al.* [7] confirming the presence of a specific nucleoside transport system in the rat intestine for Cyd and Urd without ruling out the existence of passive diffusion, the literature dealing with the transport mechanism of AraC and similar nucleosides across the intestinal wall are scant. It was, therefore, our opinion that the use of a flexible technique of isolated rat intestine with continuous perfusion for testing the permeation characteristics could help to: (1) better understanding of the mechanism(s) involved in intestinal transport of AraC and allied nucleosides, (2) elucidate the possible biotransformations of AraC analogs by intestinal tissues.

† Abbreviations: AraC, 1-β-D-arabinofuranosylcytosine; cCyd, O^2,2′-cyclocytidine; *N*-Ac-araC, *N*-acetylarabinosylcytosine; Ac_3araC, tri-*O*-acetylarabinosylcytosine; Ac_4araC, tetraacetylarabinosylcytosine; AraU, 1-β-D-arabinosyluracil; Ac_3araU, triacetylarabinosyluracil; cUrd, O^2,2′-cyclouridine; Cyd, cytidine; Urd, uridine.

MATERIALS AND METHODS

Materials. The test substances were synthesized as indicated by references [8–16], i.e. AraC, cCyd, *N*-Ac-araC, Ac_3araC, Ac_4araC, AraU, Ac_3araU, cUrd, while the natural nucleosides Cyd and Urd are commercial products (Pharma-Waldhof GmbH, Mannheim) and the structures are shown in Fig. 1. Sodium chloride, potassium chloride, calcium chloride, anhydrous disodium phosphate, dihydrogen phosphate, potassium dihydrogen phosphate and sodium bicarbonate were of analytical reagent grade.

Everted jejunal preparation and the design of permeation studies. Male albinotic rats of Wistar strain weighing 250 ± 20 g were used for the study. The animals have been fasted for 20 hr but water consumption was *ad lib*. After sacrifice the small intestine was exposed by midline incision of the abdomen. The mucosal side was thoroughly and gently washed by a flush of Krebs–Ringer bicarbonate solution [17], pH 7.4, and the proximal end of about 14 cm next to the duodenum was sleeved onto tygon cannula, ligated with silk thread and everted. After appropriate trimming a segment of 10 cm length was ligated at the distal end to another cannula. The serosal side was also washed by passing 20 ml of the perfusion fluid (Krebs–Ringer bicarbonate). The cannulated intestinal segment was immediately suspended in the organ bath of the perfusion apparatus as described elsewhere [18] but with the modification that it was connected with the outflow from a peristaltic pump (VD ČSAV SC4) on one side and to a continuous flow cuvette of an u.v. concentration monitor on the other side. The outflowing solution from the cuvette was returned back to a reservoir for recirculation.

The organ bath contained the compound under investigation at different concentrations in a final volume not exceeding 30% of the circulating volume. The incubation medium in the organ bath as well as the circulating one consisted of Krebs–Ringer

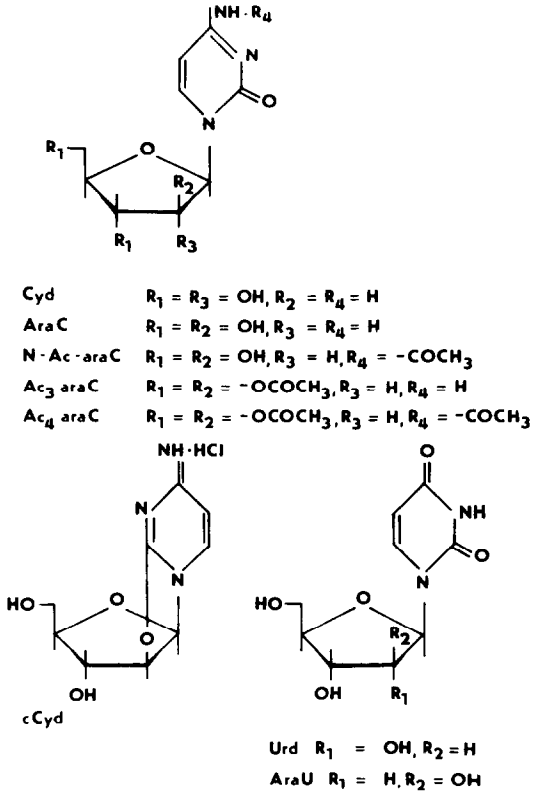

Fig. 1. Formulae of 1-β-D-arabinofuranosylcytosine and its analogs studied. Ac_3araU and cUrd, which are not included in the figure, are the deaminated metabolites of Ac_3araC and cCyd respectively.

bicarbonate. The organ bath solution represented the mucosal fluid and the circulating one was the serosal fluid. Usually the volumes of mucosal and serosal media were 30 and 100 ml respectively. Perfusion rate of serosal side *in vitro* was kept at 4 ml/min to help mixing of the transported permeant into the serosal bulk and the dead volume of the tubing system was about 5 ml. The whole system was thermostated at 37° by a universal thermostat. Oxygenation of the organ bath was done continuously by carbogen at constant rate through a glass tube with tapered end which also allowed good mixing of the mucosal fluid.

The permeated drug concentrations and consequent amounts were recorded at different time intervals by u.v. concentration monitor after setting it on the proper wavelength: 271 nm for AraC, Cyd and Ac_3araC, 250 nm for *N*-Ac-araC and Ac_4araC, 275 nm for cCyd and 260 nm for both AraU and Urd.

Testing of the functional integrity of the intestinal preparation. Functional integrity of the everted intestinal preparation in so far as nucleosides transport described herein is concerned was demonstrated in two ways. First, the intestinal preparation was incubated for 10 min with 2 initial concentrations (8.2 or 20.5 mM) of AraC at the mucosal side and the serosal drug content was assessed. Then immediately the organ bath was emptied and washed with Krebs–Ringer solution several times; meanwhile the serosal side was washed by perfusion for 10 min with the same solution or till u.v. monitor showed no detectable AraC content. Thereafter AraC in the appropriate concentration (8.2 or 20.5 mM) was added to the organ bath and AraC transport characteristics was conducted as usual. Secondly, the time course of disappearance of AraC from the intestinal mucosal side by using *in situ* technique with single circulation as described earlier [19, 20] with slight modification was followed. The timed collection of the single circulation mucosal perfusate was carried out every 5 min using intestinal segment of 20 cm length of fasted animals and perfusion rate of 0.9 ml/min. The concentration of AraC in the perfusion fluid (Krebs–Ringer bicarbonate) was 0.1 mM. The amount of AraC disappearing from the jejunal lumen every 5 min (rate) was estimated as follows:

> Rate of disappearance (amount disappearing per 5 min) = concentration of AraC in inflowing perfusion fluid × 4.5 − AraC concentration in outflowing fluid × volume of outflowing fluid.

That is to consider any change in the fluid volume during jejunal perfusion. Then the time course of AraC mucosal disappearance was compared with rates of AraC appearance in the serosal side from *in vitro* experiments.

Chromatographic analyses by HPLC. For the high performance liquid chromatography (HPLC) the equipment described recently [21, 22] was used. The liquid chromatograph consisted of a Milton Roy Model 396-57 minipump, a model 709 pulse damper, model 1203 UV III monitor (all from LDC), septum injector and a model EZ 13 electronic recorder. The reversed phase chromatography was performed on Separon S1 C 18 octadecyl-silica, 10 μm size (Laboratorní přístroje, Praha), packed in stainless steel column, 250 mm long and 4.2 mm i.d. The mobile phase was prepared from deionized water and analytical grade methanol and acetic acid. Two mobile phases composition were used to cover the widely differing mobilities of the compounds measured (parts by volume): A (the weaker eluant), 0.5 M acetic acid 90, methanol 10; B (the stronger eluant), 0.5 M acetic acid 35, methanol 65. The detection of AraC, derivatives and metabolites was performed spectrophotometrically. The retention (capacity) factors for the compounds of interest are given in Table 1.

Table 1. HPLC capacity factors (k) of AraC analogs and metabolites

Compound	Mobile phase A	Mobile phase B
AraC	0.73	—
cCyd	1.01	—
N-Ac-araC	1.5	—
Ac_3araC	—	1.77
Ac_4araC	—	0.25
AraU	0.51	—
cUrd	0.25	—
Ac_3araU	2.55	—
Cyd	0.42	—
Urd	0.3	—

Determination of the apparent coefficient, P. The partition coefficient P, was estimated according to Leo *et al.* [23] and Rekker [24]. The amount of 20 mg of the compound was dissolved in 10 ml of the aqueous or 10 ml of octanol phase. When the compound was dissolved in one phase, an equal volume of the other phase was added. The mixture was shaken in stoppered flasks at room temperature for 30 min. The separation was achieved by centrifugation at 3500 r.p.m. for 10 min. The concentration of the compound was measured both in the aqueous and octanol phases by HPLC as described above. P was calculated from the formula:

$$P = \frac{C_o}{C_a}$$

where C_o is the concentration of the test compound in the organic phase and C_a is its concentration in the aqueous one.

Drug solutions. All drugs except Ac_4araC were dissolved in the appropriate amount of the organ bath medium. When the prepared solution was added to the organ bath containing the intestinal segment the final volume was constant for a set of experiments. Ac_4araC was dissolved in the minimum amount of propylene glycol which constituted 4% of the final volume in the organ bath. Control studies were conducted to ascertain the effect of 4% propylene glycol on the permeation of other water soluble analogs which was insignificant. Concentrations of AraC and Cyd which were used in the present transport study ranged from 0.1 to 41 mM. All types of experiments were repeated at least 4 times and the mean values are presented. For comparative study across rat intestine of the analogs the concentration of 8.2 mM was chosen.

Computational scheme. Transport rates of AraC and Cyd from the mucosal to the serosal side at different initial concentrations were calculated as the ratio of the finite differences $\Delta m/\Delta t$, where Δm is the increase of the accumulated amount during the finite period Δt, Δt was considered as 60 min for the calculation of the mean flux rate, 1 min for the calculation of maximum flux rate and 30 min (between 30 and 60 min of the experiment) for the calculation of the steady state rate.

Rates as functions of initial concentrations were fitted by Michaelis–Menten equation or a combination of Michaelis–Menten with a first order process by using nonlinear regression analysis programmed in basic for minicomputer ADT 4316 [25]. The improvement of fit by including the linear parameter was tested by means of F-test according to Boxenbaum [26].

RESULTS

Sixty-minute cumulative transport of AraC and Cyd at different concentrations and transport rates across everted rat intestine. AraC and Cyd transport from mucosal to serosal side of everted rat intestine as a function of time is given in Figs. 2 and 3. It is clear that there is a rapid accumulation of AraC and Cyd during the first 10 min of the experiment in the serosal fluid followed by a much slower but almost linear process of accumulation of both compounds. Plotting the rate of transport of AraC expressed as μM/min for different concentrations as a function of time can be seen in Fig. 4. There is an initial rise in the rate of transport reaching a peak time which ranged from 3 to 10 min depending on initial concentration. The following decrease in the rate reached

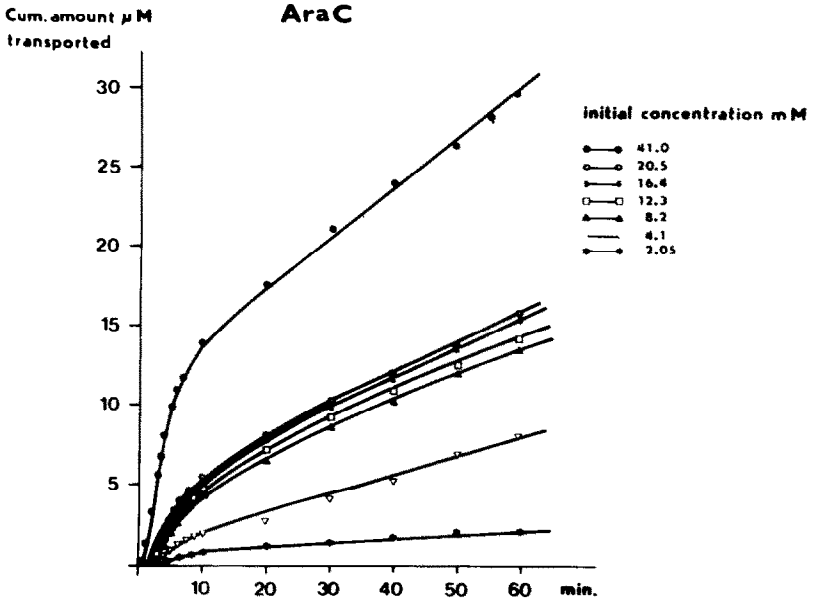

Fig. 2. Sixty-minute mucosal to serosal cumulative transport of AraC as a function of time at different initial concentrations across 10 cm of isolated, continuously perfused everted rat jejunum. Serosal and mucosal incubation media were 100 and 30 ml of Krebs–Ringer bicarbonate respectively. Samples were taken from both serosal and mucosal sides at the end of the experiments for metabolite estimation by HPLC. Values represent the mean of at least 4 experiments.

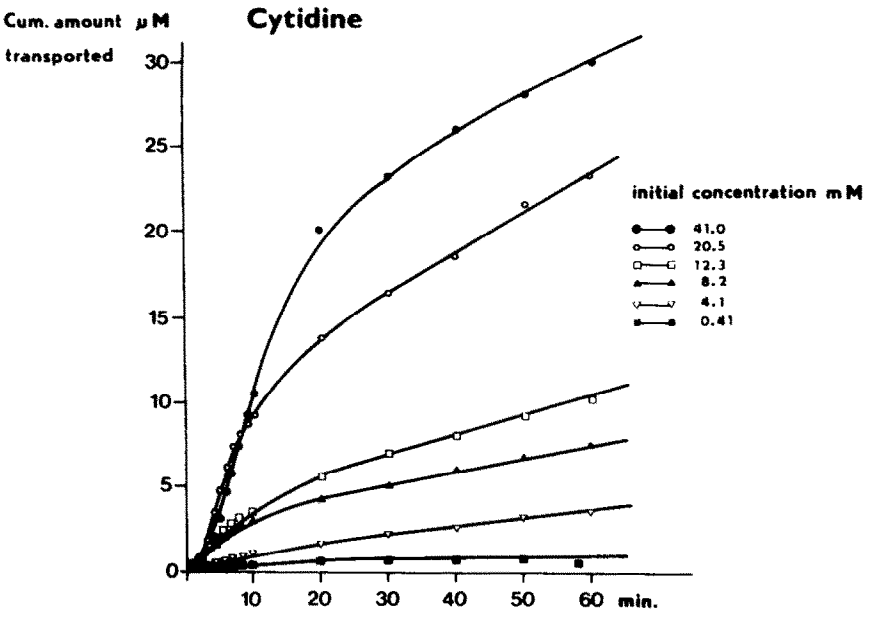

Fig. 3. Sixty-minute mucosal to serosal cumulative transport of Cyd as a function of time at different initial concentrations across 10 cm of isolated, continuously perfused everted rat jejunum. Serosal and mucosal incubation media were 100 and 30 ml of Krebs–Ringer bicarbonate respectively. Samples were taken from both serosal and mucosal sides at the end of the experiments for metabolite estimation by HPLC. Values represent the mean of at least 4 experiments.

a practically constant level between 30 and 60 min. Cyd transport rates as a function of time exhibited a similar pattern as in case of AraC.

Figure 5 shows that preincubation of the everted jejunum with AraC (8.2 mM) did not alter mucosal to serosal transport time course characteristics where it can be seen that there is similar rapid accumulation within the first 10 min in the serosal fluid followed by the slower and linear process of accumulation. This was also true when the intestinal preparation was preincubated with 20.5 mM of AraC. Moreover the results obtained from *in situ* experiments assessing the rates of disappearance of AraC from mucosal side in intact rats under the proper timed collection of the mucosal perfusate exhibited a peak rate sometime after the beginning of AraC perfusion followed

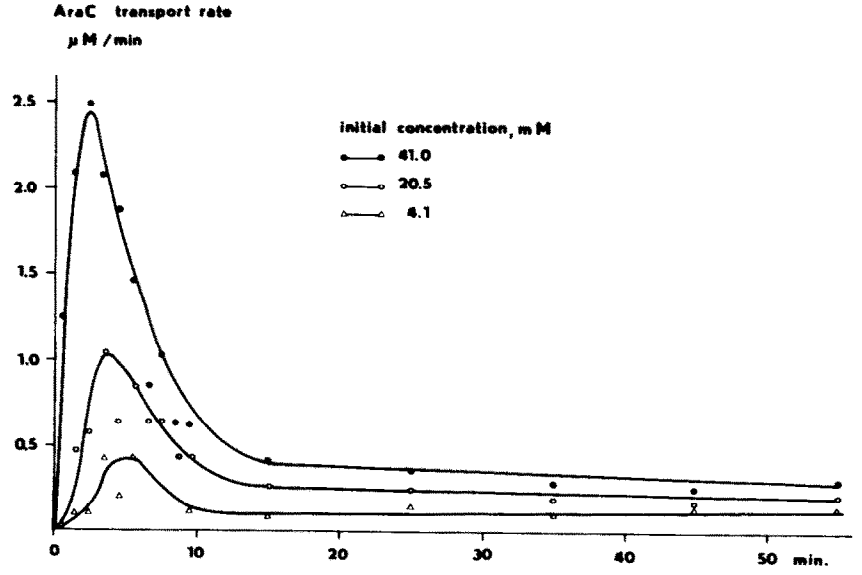

Fig. 4. Rates of AraC transport as a function of time at 3 initial concentrations across 10 cm of isolated, continuously perfused everted rat jejunum. Rate is the increase of the accumulated amount of AraC in the serosal side during the finite period of 1 min. Values represent the mean of at least 4 experiments.

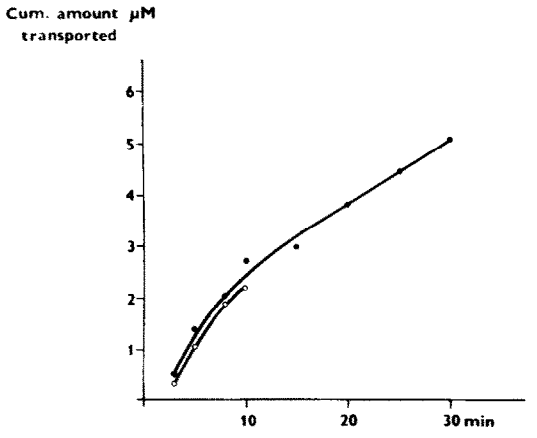

Fig. 5. Thirty-minute mucosal to serosal cumulative transport of 8.2 mM AraC as a function of time (●—●) across 10 cm of isolated, continuously perfused everted rat jejunum. The latter was preincubated in the organ bath with 8.2 mM AraC for 10 min during which the serosal cumulative drug content was assessed (○—○). Values represent the mean of 4 experiments.

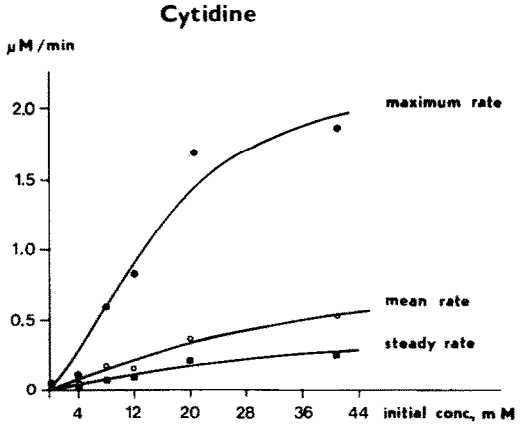

Fig. 7. Maximum, mean and steady rates of Cyd transport as a function of initial concentrations across 10 cm of isolated, continuously perfused everted rat jejunum. Values represent the mean of at least 4 experiments.

by a practically steady state rate (data not shown).

When mean, maximum and steady state rates of AraC and Cyd transport were plotted versus initial concentrations, a curvilinear behaviour was evident as illustrated in Figs. 6 and 7. In general, for both AraC and Cyd data, Michaelis–Menten equation alone or with a linear term gave the same goodness of fit. Results of fitting the data are demonstrated in Table 2 which gives Michaelis–Menten parameters with fairly good correlation coefficient.

Relationship of transport potentialities with chemical structure. The transport potentialities of a series of natural and synthetic pyrimidine nucleosides which included Urd, Cyd, AraC, AraU, cCyd, *N*-Ac-araC, Ac_3araC and Ac_4araC at single concentration (8.2 mM) in the mucosal medium was compared to relate them to the chemical structure and lipophilicity. The results of this study are summarized in Table 3. Considering uridine as the basic compound, the effect of the following modification in chemical or sterical structure could be evaluated from these comparisons:

(a) Modification of the pyrimidine nucleus

1. Substitution of the pyrimidine hydroxyl by amino group transforming Urd to Cyd and AraU to AraC decreases lipophilicity but increases transport rate. The increase in transport can be considered as additive since the increment is practically the same for both comparisons.

2. *N*-Acetylation of the amino group has an opposite effect in both respects leading to an increase in lipophilicity but to a decrease in transport rate. This is evident from the comparisons *N*-Ac-araC vs AraC and Ac_4araC vs Ac_3araC. However, in contrast to the preceding modification the effect of *N*-acetylation is clearly nonadditive.

(b) Modifications of the sugar moiety

1. A shift of 2′-OH group from the *cis* to the *trans* (with regard 3′-OH group) position which transforms Urd to AraU and Cyd to AraC results in an increase in lipophilicity accompanied by a parallel increase in transport rate. Again, the change in transport rate can be considered as additive.

2. Acetylation of arabinose moiety increases lipophilicity, but decreases transport rate. This can

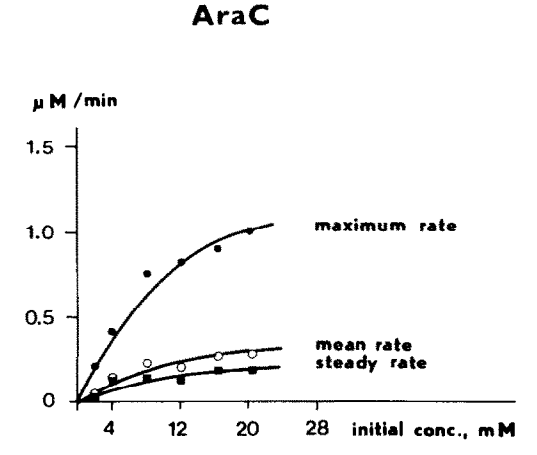

Fig. 6. Maximum, mean and steady rates of AraC transport as a function of initial concentrations across 10 cm of isolated, continuously perfused everted rat jejunum. Values represent the mean of at least 4 experiments.

Table 2. Michaelis–Menten parameters and correlation coefficient (r)

	$V_m \pm$ S.D. (μM/min)	$K_m \pm$ S.D. (μM/ml)	r
AraC			
Mean rate	0.36 ± 0.07	7.9 ± 3.6	0.96
Steady rate	0.23 ± 0.06	5.9 ± 3.9	0.86
Maximal rate	1.6 ± 0.19	11.4 ± 2.9	0.99
Cyd			
Mean rate	0.84 ± 0.3	30.7 ± 21.0	0.96
Steady rate	0.38 ± 0.12	24.0 ± 14.0	0.96
Maximal rate	2.9 ± 1.0	23.0 ± 15.0	0.95

Table 3. Transport rates across everted rat jejunum, partition coefficient (log *P*) and the metabolites appearing in the serosal side

Compound	Transport rate (μM/hr)*	Partition coefficient (log *P*)	Percent of parent compound and metabolites Compound	Percent
AraC	13.6 ± 0.8	−2.05	AraC	85
			AraU	15
N-Ac-araC	4.3 ± 0.7	−1.35	*N*-Ac-araC	87.5
			AraC	7
			AraU	5.5
Ac_3araC	6.8 ± 1.5	−0.8	Ac_3araC	65
			Ac_3araU	35
Ac_4araC	4.0 ± 0.2	−0.16	Ac_4araC	45
			Ac_3araU	39
			N-Ac-araC	16
cCyd	9.2 ± 1.1	−4.03	cCyd	62
			cUrd	29
			AraU	9
Cyd	9.0 ± 2.1	−2.51	Cyd	97.5
			Urd	2.5
AraU	11.0 ± 0.4	−1.71	no change	
Urd	6.2 ± 0.9	−1.98	no change	

* Mean of at least 4 experiments ± S.D.

be documented by the comparisons Ac_3araC vs AraC as well as Ac_4araC vs *N*-Ac-araC. However, similarly as with *N*-acetylation the extent of change in transport rate is rather nonadditive depending on the rest of the molecule.

(c) Formation of internal anhydroring between the arabinose and pyrimidine nucleus which results in transformation of AraC to cCyd is accompanied by parallel decrease of lipophilicity and of transport rate.

It is clear from these comparisons that alterations in transport potentiality do not go parallel with changes in the lipophilicity of the compounds and that modifications in chemical or even steric structure appear to be more decisive in this respect. For instance, the importance of a free amino group in position 4 of pyrimidine nucleus is most striking.

Biotransformation of nucleosides during penetration of the intestinal wall. The biotransformation activity of the intestinal wall towards penetrating nucleosides can be judged from the results of HPLC analyses of the serosal fluid performed at the end of the experiment. These results which are summarized in Table 3 show that deamination activity converting AraC to an inactive product AraU does exist in the intestinal wall. It is noteworthy, however, that the natural compound Cyd was more resistant to deamination than AraC. On the other hand, further changes in the chemical structure of AraC e.g. acetylation of the sugar moiety as well as ring closure between the sugar moiety and the pyrimidine nucleus resulted in an increased proportion of deaminated products which was roughly of the same extent in both cCyd and Ac_3araC. With *N*-acetylated compounds (*N*-Ac-araC and Ac_4araC), this proportion was much smaller with *N*-Ac-araC (5.5%) with Ac_4araC similar to Ac_3araC (39%). But considering that deamination of *N*-acetylated aminonucleosides is evidently dependent on the extent of *N*-deacetylation, which was 12.5% in *N*-Ac-araC and 39% in Ac_4araC, the "true" deamination rate must be in fact higher, 44% in the former and 100% in the latter compound. As far as *O*-deacetylation is concerned it seems that the capacity of the intestinal wall to cleave the *O*-acetyl bond is slightly less as we found only 16% of *N*-Ac-araC formed from Ac_4araC but no AraC formed from Ac_3araC.

DISCUSSION

The present technique adopted for assessing the permeation of compounds across rat intestine permitted accurate and continuous evaluation of not only the transport behaviour of the compound under investigation but in combination with HPLC technique also the metabolic alterations that took place to the analogs by the intestinal tissues in the incubation medium. Everted jejunum within the present incubation conditions has proved to keep its functional integrity with regard to AraC transport. This has been ascertained by preincubation with high AraC concentrations where the intestinal preparation exhibited the typical apparent biphasic serosal cumulation of the drug. The time course of appearance of the nucleoside in the serosal side has been confirmed also by the results obtained from our *in situ* experiments assessing the rates of disappearance of AraC from the mucosal side in intact rat under appropriate timed collection of the perfusate where both exhibited a similar profile. However specific rate limiting barriers for drugs within the epithelium have not been well identified. Therefore the mucosa has been treated as "black box" with regard to structures that constitute the barrier, even though the anatomical details of this tissue are well known as well as the histological alterations that occur usually during *in vitro* experiments are well documented [27–30]. Apart from the penetration of the nucleosides through the intestinal barrier(s), several reports are dealing with nucleosides transport in different mammalian cell lines or isolated perfused mouse liver [31–35]. In all those studies it was proved that many nucleosides enter cells by the way of the nucleoside-specific transport mechanism(s). It seems that this is

also true with regard to intestinal transport. The process of transport is saturable which is supported by fairly good fitting of the flux rates by Michaelis–Menten equation.

Moreover the dependence of the peak and time of initial transport rate on mucosal concentration to certain extent may be suggestive, too, for a limited number of "nucleosides-carrier" sites working between mucosal and serosal sides in a round trip. Mathematical modelling of the nucleosides transport characteristics as revealed from the results and the simulation by the computer showed relevance between experimental data and the theoretical model including a carrier system for the nucleosides and this will be reported later on.

The concentrations which may reflect the affinity of the compounds AraC or Cyd to the carrier system at half the maximum velocity of transport, K_m, are relatively high. Therefore it is questionable whether this might indicate a true low affinity. Histologically, the intestinal wall is composed of mucosa with its well-known superficial epithelium, an underlying stroma composed of vascularized, highly cellular reticular connective tissue (lamina propria), the submucosa, the muscularis and serosa [36]. Hence the transport across intestinal potential barrier(s) is a multistep process. Thus K_m estimates obtained here represent rather an operational parameter serving for comparison of different compounds under identical conditions but which hardly can be compared with values of K_m found with isolated cell systems. Nevertheless, Wohlheuter *et al.* [37] investigating thymidine transport in cultured mammalian cells obtained 200-fold the K_m of thymidine uptake described by other investigators.

The pH-partition theory [19, 20] predicts that unionized forms of the foreign compounds should penetrate the boundary between blood and intestine by passive diffusion at rates related to their lipid solubilities. Contrarily, in the present investigation the fact that alterations in transport rates did not go parallel with lipid solubility of the compounds is not consistent with the simple passive diffusion assumption. On the other hand, it seems that the free amino group is of utmost importance in a carrier-mediated transport process. This is also evidenced by the slightly less transport rate exhibited by AraU and Urd as compared with the corresponding aminonucleosides. Similarly, it was found [38] that a free α amino group is essential for intestinal absorption of amino acid derivatives.

The experiments performed in the present study gave us the opportunity to demonstrate that during the incubation of the intestinal segment pyrimidine nucleosides are subjected to various biotransformation reactions. The deamination reaction observed by us is, however, not consistent with the findings of Camiener and Smith [39] who under conditions of gross contamination by intestinal bacteria described only negligible deaminase activity by rat liver homogenates. Owing to the sensitive HPLC method which is able to detect very low concentration of metabolite(s) we were able to report for the first time deaminase activity towards AraC and analogs in the rat small intestine. If this type of deaminase activity is specific for the rat gut, or if it will be detectable also in other biological compartments as blood, liver or kidney when sensitive techniques are employed for metabolite detection, remains to be established. Nevertheless, AraU was formed in the urine of rats [5] after oral AraC. With respect to the results of Camiener and Smith [39], the transformation was explained by the activity of intestinal microflora. However, it is unlikely to be the case in the present investigation, since the deaminated as well as the deacetylated products were detected only in the serosal side of the perfused intestinal segment. Our unpublished data showed that there was no measurable AraU by HPLC in rats blood after intravenous 50 mg/kg of either AraC or cCyd. It seems, therefore, that the detectable deaminase activity is confined mainly to the intestine. Moreover, El Dareer *et al.* [40] reported a high concentration of cCyd and the metabolites AraC and AraU in the small intestine relative to liver and other tissues after intraperitoneal and oral doses. Deacetylation reactions are in agreement with the results of our previous report [41] where it was concluded that there are nonspecific esterases in the epithelium of intestinal mucosa.

In conclusion AraC analogs translocation across rat jejunum is a carrier mediated process, and in general the rate of transport is not consistent with the pH-partition theory of membrane transport. The transport rates and metabolic transformations of different analogs is dependent on the chemical structure of the nucleoside. Moreover, a free amino group of cytosine moiety increased the rate of transport within the present series of AraC analogs. There were detectable deaminase as well as esterase activities toward AraC and its analogs in rat jejunum.

REFERENCES

1. E. Frei, J. N. Bickers, J. S. Hewlett, M. Lane, W. V. Leary and R. W. Talley, *Cancer Res.* **29**, 1325 (1969).
2. G. P. Bodey, E. J. Freireich, R. W. Monto and J. W. Hewlett, *Cancer Chemother. Reports* **53**, 59 (1969).
3. J. A. Streifel and S. B. Howell, *Proc. natn. Acad. Sci. U.S.A.* **78**, 5132 (1981).
4. D. H. W. Ho and E. Frei, *Clin. Pharmac. Ther.* **12**, 944 (1971).
5. O. Yusumichi, T. Nobuklyo, O. Atsuhiku and K. Jushikazu, *Jakugaku Zasshi* **92**, 592 (1972).
6. A. R. Hanze and G. W. Camiener, U. S. 4,017,606 (CL. 424-18, A61 K31/70), 12 Apr. (1977).
7. G. Sciorelli, U. Ventura and G. Grignani, *Boll. Soc. Ital. Biol. Sper.* **45**, 1538 (1969).
8. J. Beránek, J. Brokeš and H. Hřebabecký, *Czech.* 1502 (1977).
9. J. Beránek, T. J. Delia and P. Drašar, *Collect. Czech. Chem. Commun.* **42**, 1588 (1977).
10. J. Beránek, J. Brokeš and H. Hřebabecký, *Czech.* 1501 (1977).
11. J. Beránek, T. J. Delia and P. Drašar, in *Nucleic Acid Chemistry* (Eds. L. B. Townsand and R. S. Tipson), p. 249. Wiley-Interscience, New York (1978).
12. J. Brokeš and J. Beránek, *Czech. Chem. Commun.* **39**, 3100 (1974).
13. K. A. Watanabe and J. J. Fox, *Angew Chem.* **78**, 589 (1966).
14. J. Beránek and P. Drašar, *Collect. Czech. Chem. Commun.* **31**, 366 (1977).

15. A. P. Martinez, W. W. Lee and L. J. Goodman, *J. med. Chem.* **9**, 268 (1966).
16. J. P. H. Verheyden, D. Wagner and J. G. Moffat, *J. org. Chem.* **36**, 250 (1971).
17. W. W. Umbreit, R. H. Burris and J. F. Stauffer, *Manometric Techniques,* 2nd print, p. 135. Burgess Publishing Co., Minneapolis (1959).
18. D. Terziivanov, I. Janků and I. Havlík, *J. Pharm. Pharmac.* **34**, 817 (1982).
19. L. S. Schanker, D. J. Tocco, B. B. Brodie and C. A. M. Hogben, *J. Pharmac. exp. ther.* **123**, 81 (1958).
20. C. A. M. Hogben, D. J. Tocco, B. B. Brodie and L. S. Schanker, *J. Pharmac. exp. ther.* **125**, 275 (1959).
21. M. Ryba, *J. Chromat.* **219**, 245 (1981).
22. M. Ryba and J. Beránek, *J. Chromat.* **211**, 337 (1981).
23. A. Leo, C. Hansch and D. Elkins, *Chem. Rev.* **71**, 525 (1971).
24. R. F. Rekker, *The Hydrophobic Fragmental Constant.* Elsevier, Amsterdam (1977).
25. I. Janků, Package of pharmacokinetic programs for minicomputer ADT 4316 (1981).
26. G. Boxenbaum, S. Riegelman and R. M. Elashoff, *J. Pharmacokin. Biopharm.* **2**, 123 (1974).
27. R. R. Levine, W. F. McNary, P. J. Kornguth and R. LeBlanc, *Eur. J. Pharmac.* **9**, 211 (1970).
28. L. Z. Benet, J. M. Orr, R. H. Turner and H. S. Webb, *J. Pharmac. Sci.* **60**, 234 (1971).
29. M. Gibaldi and B. Grundhofer, *J. Pharmac. Sci.* **61**, 116 (1972).
30. Z. T. Chowhan and A. A. Amaro, *J. Pharmac. Sci.* **66**, 1249 (1977).
31. J. M. Zylka and P. G. W. Plagemann, *J. biol. Chem.* **250**, 5756 (1975).
32. J. H. Mulder and K. R. Harrap, *Eur. J. Cancer* **11**, 373 (1975).
33. A. R. P. Paterson, N. Kolassa and C. E. Cass, *Pharmac. Ther.* **12**, 515 (1981).
34. E. R. Harley, A. R. P. Paterson and C. E. Cass, *Cancer Res.* **42**, 1289 (1982).
35. N. Kolassa, and A. R. P. Paterson, *Can. J. Physiol. Pharmac.* **60**, 167 (1981).
36. W. L. Hayton, *J. Pharmacokin. Biopharm.* **8**, 321 (1980).
37. R. M. Wohlheuter, R. Marz and P. G. W. Plageman, *Biochim. Biophys. Acta* **553**, 262 (1979).
38. G. L. Amidon, M. Chang, D. Fieisher and R. Allen, *J. Pharmac. Sci.* **71**, 1138 (1982).
39. G. W. Camiener and C. G. Smith, *Biochem. Pharmac.* **14**, 1405 (1965).
40. S. M. El Dareer, V. M. White, K. Tillery, F. P. Chen, L. B. Mellet and D. L. Hill, *Cancer Treatment Reports* **61**, 805 (1977).
41. J. Plevová, H. M. Farghali and I. Janků, *Biochem. Pharmac.* **20**, 2079 (1971).