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ARPRINOCID, AN INHIBITOR OF HYPOXANTHINE–GUANINE TRANSPORT

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Abstract—Arprinocid showed no appreciable effect on hypoxanthine-guanine phosphoribosyl transferase, but demonstrated competitive inhibition of transport of hypoxanthine ($K_I = 33\,\mu M$) and guanine ($K_I = 79\,\mu M$) in HeLa cells. The drug had little effect on the transport of adenine which was mediated apparently by a separate carrier system in HeLa cells. Analogs of arprinocid were synthesized and tested on hypoxanthine transport. The inhibitory activities correlated reasonably well with the *in vivo* anticoccidial activities which led to the conclusion that the mode of anticoccidial action of arprinocid could be by inhibiting hypoxanthine transport in the parasite. The drug had relatively high affinity to HeLa cells and *Eimeria tenella* sporozoites but was poorly absorbed to phosphatidyl choline liposomes. The difference was attributed to possible high affinity binding between the drug and the hypoxanthine-guanine carrier in cell membranes.

In a previous communication [1], we indicated that arprinocid, 6-amino-9-(2-chloro-6-fluorobenzyl) purine (I), inhibited hypoxanthine-guanine salvage in HeLa cells. There was also an indication that a similar effect was exerted on the parasite *Eimeria tenella* [1], which could account for the mode of anticoccidial activity of the drug because coccidia probably lack the ability of *de novo* purine synthesis [1].

There are at least two biochemical events involved in the salvage pathway of hypoxanthine and guanine by a mammalian cell: transport of purines [2–6] and the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) which converts the purines and PRPP to corresponding ribonucleotides [7]. The mechanism of transport is most likely facilitated diffusion [5] and has just begun to be understood. Azaguanine-resistant mutants of Chinese hamster ovary cells have been isolated in which the HGPRT activity appeared normal [4]. A similar temperature-sensitive mutant with normal HGPRT exhibited a temperature-dependent alteration in the transport of hypoxanthine, guanine, 8-azaguanine, and guanosine but not of adenine, adenosine or thymidine [2]. This alteration in transport appeared to be a recessive genetic trait [2]. Further studies clearly indicated the presence of two separate, carrier-mediated purine transport systems in Chinese hamster lung fibroblasts [3] and Novikoff rat hepatoma cells [5]. They operated independently of phosphoribosylation, and functioned in the HGPRT-deficient mutants [3, 5]. One system specifically transported hypoxanthine and guanine whereas the other was specific for adenine transport [3, 5]. The apparent K_m values for purine transport were higher, and the apparent V_{max} values were lower than those for the corresponding phosphoribosyl transferases [5], which suggest that the transport might be the rate-limiting step in the purine salvage pathway.

In our previous studies there was some preliminary indication that arprinocid had no effect on the HGPRT activity in crude extracts of chicken liver [1]. It was the purpose of the present investigation to see if the drug also lacks effect on the HGPRT in HeLa cells, and to test if arprinocid acts as a specific inhibitor of hypoxanthine-guanine transport in HeLa cells. Confirmation of the latter has led to studies of the possible correlation between inhibition of hypoxanthine-guanine transport in HeLa cells and the *in vivo* anticoccidial activity among many analogs of arprinocid. The results support the view that arprinocid (I) may act on coccidia by blocking hypoxanthine transport.

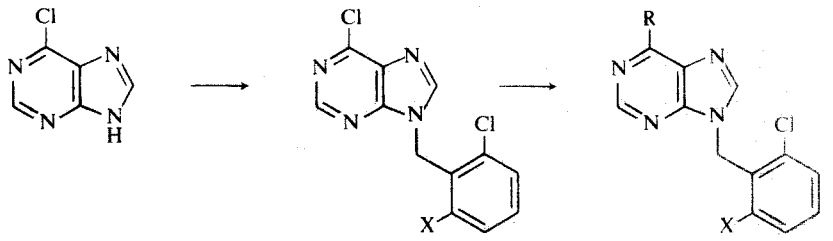
MATERIALS AND METHODS

Materials

HeLa S3 cells and *E. tenella* sporozoites were prepared as described previously [1]. Labeled materials were obtained from New England Nuclear, Boston, MA, whereas other chemicals were of the highest purity available from commercial sources. Analogs of arprinocid were synthesized by the method described in Scheme I.

The electron-withdrawing effect of the 6-chloro substituent upon the *N*-3 nitrogen of purine favored the *N*-9 benzylation of 6-chloropurine, which was accomplished in the presence of sodium hydride and *N*,*N*-dimethylformamide. Chloride ion underwent facile displacement by a variety of substituted amines and other strong nucleophiles to furnish I and other 6-substituted purine derivatives (IV-XXIII) according to the following general procedures. Satisfactory elemental analyses (C, H, N) and mass spectra were obtained for all compounds.

6-Chloro-9-(2,6-dichlorobenzyl)purine (III). Sodium hydride (57% oil dispersion, 4.0 g) was slurried with petroleum ether (3 × 100 ml) and the organic solvent was carefully decanted and discarded. To the residue was added dimethylformamide (125 ml) and then carefully with cooling 6-chloropurine (12.0 g). When the effervescence and foaming had subsided, α,2,6-trichlorotoluene (20.0 g) was added and the sealed flask was stirred at room temperature. Salt began



II X = F
III X = Cl

I R = NH_2, X = F
IV R = OH, X = F
V R = SH, X = F

VI R = $OCH(CH_3)_2$, X = Cl

VII R = $N(CH_3)_2$, X = F
VIII R = $N(CH_3)_2$, X = Cl
IX R = $NHCH_2CH_3$, X = Cl
X R = $N(CH_2CH_3)_2$, X = Cl
XI R = $NHCH(CH_3)_2$, X = Cl
XII R = $NH(CH_2)_2CH_3$, X = Cl
XIII R = $NH(CH_2)_3CH_3$, X = F

XIV R = $NH(CH_2)_3CH_3$, X = Cl
XV R = $NH(CH_2)_4CH_3$, X = Cl
XVI R = $NH(CH_2)_5CH_3$, X = Cl

XVII R = N⟨ ⟩, X = Cl

XVIII R = $N(CH_2)_3CH_3)_2$, X = Cl
XIX R = $NHCH_2C_6H_5$, X = F
XX R = $N(CH_2CH_2OH)_2$, X = F
XXI R = $NCH_2CH_2OCH_2CH_2$, X = Cl
XXII R = $NHN(CH_3)_2$, X = Cl
XXIII R = $NH(CH_2)_2N(CH_3)_2$, X = Cl

to separate from the clear solution within minutes. After 24 hr the solids were filtered and washed with dichloromethane and discarded. The volume of the filtrate and washes was reduced *in vacuo* at less than 40° to an approximate volume of 25–30 ml and diethyl ether was added (100 ml). The resultant crystalline mass that formed over a period of several hours was filtered, washed with a small amount of ether, and air-dried (15.1 g, 62%).

The 6-fluoro analog (II) was prepared in 55% yield by the method for the preparation of II using 2-chloro-6-fluorobenzyl chloride.

9-(2-Chloro-6-fluorobenzyl)purin-6(1H)-thione (V). A round bottomed flask was charged with thiourea (0.1 g), 10% aqueous ethanol (10 ml) and II (0.2 g) and heated at reflux for 18 hr. The reaction mixture was evaporated to dryness and extracted with water. The insoluble material was recrystallized from methanol to give V as an off-white solid (0.185 g, 93%).

6-Isopropoxy-9-(2,6-dichlorobenzyl)purine (VI). Sodium hydride (57% oil dispersion, 0.1 g) was washed with petroleum ether (3 × 50 ml) to remove mineral oil and isopropanol (20 ml) was added. After solution had been achieved (15 min), III (0.4 g) was added and the mixture was stirred at room temperature for 48 hr. Acetic acid was added to neutralize the remaining base and the volatiles were removed by evaporation under a stream of nitrogen. The resultant solid was suspended in water, filtered, and then recrystallized from aqueous methanol to furnish XXI as colorless crystals (0.42 g, 97%), m.p. 98–100°.

Compound IV was prepared by an analogous procedure using alcoholic sodium hydroxide solution.

6-Butylamino-9-(2,6-dichlorobenzyl)purine (XIV). 6-Chloro-9-(2,6-dichlorobenzyl)purine (III) (0.75 g) was covered with 10 ml anhydrous *n*-butylamine, sealed, and stirred magnetically for 48 hr at room temperature. The reaction was complete at that time according to the t.l.c. monitoring system, dichloromethane–methanol (19:1) on silica gel. The amine was evaporated under a stream of nitrogen and the residue was stirred for 30 min in 100 ml water, filtered, washed with water and air-dried. Recrystallization from dichloromethane–petroleum ether (30:60) gave colorless crystals (0.79 g, 94%), m.p. 166°.

Other analogs prepared by the above method included I (m.p. 166°), VII (m.p. 136–137°), VIII (m.p. 177–178°), IX (m.p. 214–216°), X (m.p. 143–145°), XI (m.p. 99–100°), XII m.p. 206–208°), XIII (m.p. 136–137°), and XXII (m.p. 217°, shrinks 190°).

6-Di-n-butylamino-9-(2,6-dichlorobenzyl)purine (XVIII). To III (0.5 g) in a round-bottomed flask was added di-*n*-butylamine (10 ml) and the stoppered flask was stirred magnetically for 48 hr until the reaction was complete by t.l.c. monitoring dichloromethane–methanol (19:1) on silica gel.

The solvent was evaporated under high vacuum and the residue was dissolved in dichloromethane and extracted with dilute acetic acid (3 × 150 ml) until the water layer was pH 1, and then with saturated salt solution until neutral. The organic layer was dried over sodium sulfate and evaporated to a gum which was crystallized from aqueous methanol to give colorless plates (1.39 g, 60%) m.p. 87–88

Other analogs prepared by this method included XV (m.p. 126°), XVI (m.p. 121°), XVII (m.p. 186–187°), XIX (m.p. 220–221°), XX (m.p. 124–126°), XXI (m.p. 180–181°), and XXIII (m.p. 124°).

Methods

Biological assay of *in vivo* anticoccidial activities has been described previously [1]. Hypoxanthine-guanine phosphoribosyl transferase (HGPRT) was assayed according to the procedure by Schmidt *et al.* [8], using PRPP and [^{3}H]hypoxanthine (8 Ci/m-mole) or [^{3}H]guanine (114 mCi/m-mole) as substrates. The enzymic reaction was terminated by adding NH_2CH_2COOH, and the mixture was filtered through a

PEI cellulose mat on a GFC glass fiber filter to trap the nucleotides. Radioactivity absorbed to the washed and dried mat was soaked in a small aliquot of water, mixed in Aquasol-2 (New England Nuclear) and counted in a Packard Tri-Carb liquid scintillation spectrometer.

The assay of purine transport in HeLa cells was carried out as follows. Log phase HeLa cells were washed and suspended in Eagle's minimal essential medium (EMEM) to a final concentration of 5×10^6 cells/ml. Substrates were added at various concentrations, and the cell suspension was incubated at 37°. Aliquots of 0.2 ml were taken after different time intervals, ranging from 0 to 10 min, transferred to microfuge tubes half filled with a mixture of 84% Silicone fluid 550 (Dow) + 16% heavy mineral oil (Squibb) [9], and the cells were immediately pelleted through the oil phase by centrifugation in the microfuge. The entire operation took about 5 sec. The tip of the centrifuge tube containing the pellet was cut off, the residual oil was drained, and the pellets were dissolved in 0.5 N NaOH. After being neutralized by HCl, the radioactivity in the digest was recorded in a scintillation spectrometer. The rate of uptake during the first 4 min of incubation was taken as the initial rate of uptake.

Phosphatidyl choline (PC) multilamellar vesicles (liposomes) were prepared by dissolving egg yolk phosphatidylcholine in $CHCl_3$, dried by rotary evaporation and left under high vacuum overnight. A solution of 10 mM Tris–Cl pH 7.5, and 100 mM KCl was added to give a lipid concentration ranging from 1 to 30 mg/ml. Glass beads of 3 mm diameter were added and the mixture was vortexed for 3 min to produce the liposomes.

RESULTS

Lack of effect of arprinocid on HGPRT of HeLa cells

The HGPRT activity in crude extracts of HeLa cells was assayed in the presence of arprinocid or 8-azaguanine, a weak inhibitor of HGPRT [10]. The reaction mixtures contained 10% glycerol formal (Centerchem. Inc., New York, NY) to allow solubilization of arprinocid up to 0.5 mM without appreciable solvent effect on the enzyme activity. The results, presented in Fig. 1, indicate little or no inhibitory effect on the enzyme activity by 0.5 mM arprinocid.

Inhibition of purine transport in HeLa cells by arprinocid

Kinetic studies on the transport of labeled hypoxanthine, guanine and adenine in HeLa cells and effects of arprinocid on the transport resulted in the accumulation of vast amounts of data. They are partly presented in Fig. 2, 3 and 4. The rates of uptake during the initial 4 min of incubation are approximately linear for all three substrates. Extrapolation to zero results in near zero values in all the experiments. The data from individual experiments are highly reproducible. More detailed analyses on additional data have shown that arprinocid was a potent competitive inhibitor with hypoxanthine ($K_I = 33\ \mu M$, Fig. 5) and guanine ($K_I = 79\ \mu M$, Fig. 6) but was only slightly inhibitory on adenine ($K_I = 4.4$ mM, Fig. 7). Further investigation of hypoxanthine transport revealed that guanine acted as a competitive inhibitor with a K_I value of 111 μM (Fig. 8), whereas adenine acted as a weak uncompetitive inhibitor (Fig. 9). Transport of inosine and guanosine was also inhibited by arprinocid, but the inhibitory activities were only 50 per cent of those on transport of hypoxanthine and guanine.

Correlation between inhibition of hypoxanthine transport in HeLa cells and anticoccidial activities among arprinocid analogs

Twenty-two analogs of arprinocid were synthesized and tested and the potency of each compound in inhibit-

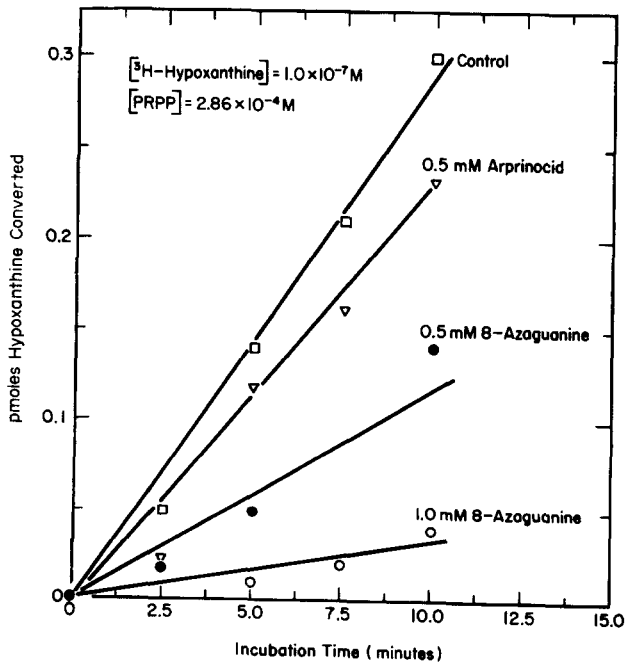


Fig. 1. Assay of hypoxanthine-guanine phosphoribosyl transferase activity in crude extracts of HeLa cells. Effects of 8-azaguanine and arprinocid on the enzyme were tested at indicated concentrations.

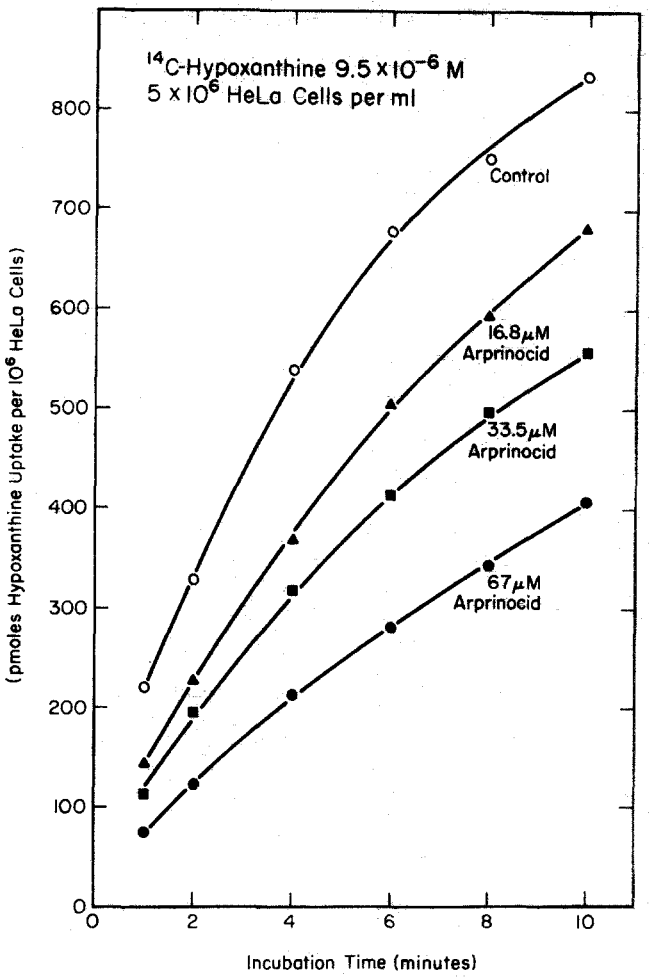


Fig. 2. Inhibition of hypoxanthine transport in HeLa cells by arprinocid. [^{14}C]hypoxanthine (53 mCi/m-mole) was used as substrate. Concentrations of arprinocid are indicated in the figure.

ing hypoxanthine uptake was compared with its anticoccidial activity assayed *in vivo*. The *in vivo* tests were run at a single dose of each drug (100 μg/gm of chick's diet) because of limitations in the quantities of the drugs. The data are thus, at best, qualitative. The final results, summarized in Table 1, exhibit reasonably good correlations between the two activities. It is also apparent from the data that alkyl-substitution at the 6-amino position of arprinicod enhances the inhibitory effect on hypoxanthine transport as well as the anticoccidial activity. However, as the alkyl group became longer, the anticoccidial activity decreased (the *n*-butyl derivatives) and was followed by a decrease also in the inhibitory activity on hypoxanthine transport (the *n*-pentyl and *n*-hexyl derivatives). It should be noted that those *in vivo* "inactive" outcomes in Table 1 could also mean "active" but less active than arprinocid. No thorough analysis of the correlation can be done until more quantitative *in vivo* data are available.

Arprinocid-1-*N*-oxide was also tested in the transport assays. It proved to be an inhibitor of hypoxanthine-guanine transport half as potent as arprinocid.

Uptake of arprinocid by HeLa cells, E. tenella sporozoites and PC liposomes

The transport of [^{3}H]arprinocid in HeLa cells was assayed by the same procedure described for purine transport. The data indicate that the drug was apparently taken up by HeLa cells at an exceedingly fast rate; equilibrium was attained within the first minute of incubation. No kinetic study was possible using the present technique; only the relationship between the concentration of arprinocid in the medium and arprinocid uptake was measured; this is presented in Fig. 10. A linear relation exists between the exogenous concentration and the amount taken up by HeLa cells up to the saturating level of arprinocid in 1% DMSO aqueous solution (67 μM). Assuming that each HeLa cell has a volume of 3.3 × 10^{-9} ml [11, *], the concentration of

$$*P = \frac{Cc - Cs\,(1-m)}{Cs\,m}$$

where Cc = concentration of drug before membrane addition; Cs = concentration of drug after membrane addition; and m = weight fraction of membranes in incubation mixture

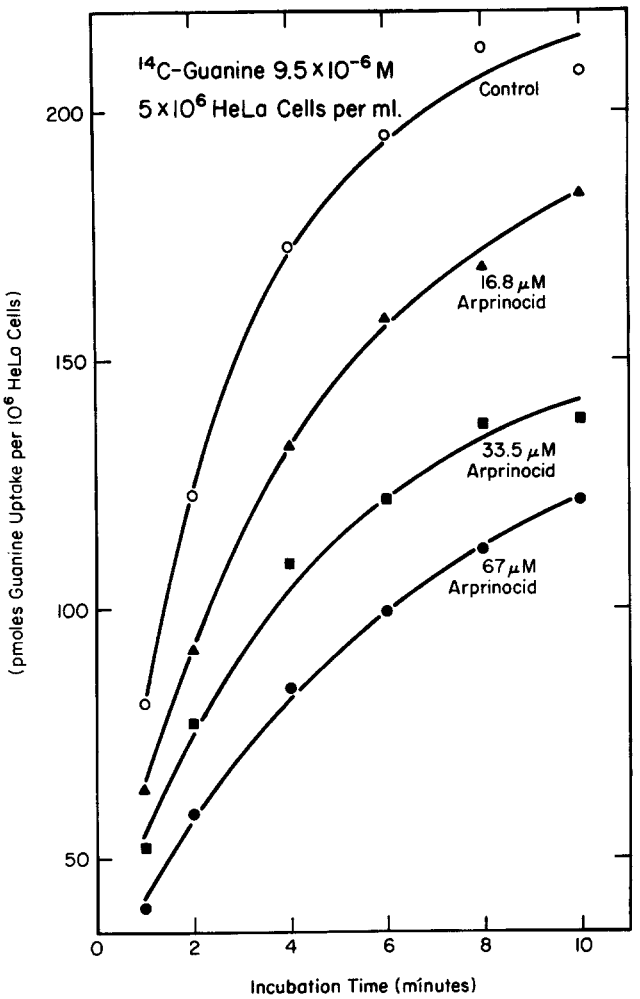


Fig. 3. Inhibition of guanine transport in HeLa cells by arprinocid. [^{14}C]Guanine (45 mCi/m-mole) was used as substrate.

arprinocid associated with the cells was calculated to be about seven times higher than that in the solution.

The uptake of [^{3}H]arprinocid by freshly purified *E. tenella* sporozoites was conducted in Ringer's phosphate, pH 7.4, containing 10^{-5} M of the drug. After different time intervals of incubation at 37°, aliquots were filtered through a Millipore, washed and dried. The radioactivities were counted. The results indicate that the uptake reached its plateau within minutes, and there were 900 pmoles arprinocid taken up by 10^{6} sporozoites. Similar experiments using [^{3}H]adenine or [^{3}H]adenosine as substrates indicated little uptake: $<$1 pmole adenine and 11 pmoles adenosine were found associated with 10^{6} sporozoites after 1 hr of incubation. Low levels of hypoxanthine transport were also observed in sporozoites, which may be due to the fact that during this extracellular phase the parasites are highly impermeable [12] and inert in nucleic acid metabolism [13]. It was thus technically not feasible to study the effect of arprinocid on purine transport in *E. tenella* sporozoites.

PC liposomes were suspended in 10 mM Tris–Cl, pH 7.5, + 100 mM KCl at concentrations ranging from 0.001 to 10 mg PC/ml and incubated with [^{3}H]arprinocid at room temperature for 3.5 hr. Aliquots of 0.1 ml volume were transferred to cellulose nitrate airfuge tubes and centrifuged in a Beckman airfuge at 100,000 *g* for 10 min. Radioactivity in the supernatant fraction was counted. The results are presented in Fig. 11 and show appreciable absorption of the drug to liposomes only when the concentration of the latter reaches 1.0 to 10.0 mg PC/ml. Partition coefficients (*P*)* [14] calculated from the data give an average value of 58 ± 12. When the liposomes were prepared at 30 mg PC/ml by hydrating with [^{3}H]arprinocid at 5 and 10 μg/ml overnight, the binding between the drug and the liposomes indicated partition coefficients of 119 and 118, respectively, at room temperature. The values became 82.6 and 94.6 when the preparations were assayed 1 month later. When the incubation was carried out at 37° for 3.5 hr, the partition coefficients turned out to be 78.2 and 88.1. The

* P. M. Simashkevich and C. C. Wang, unpublished results.

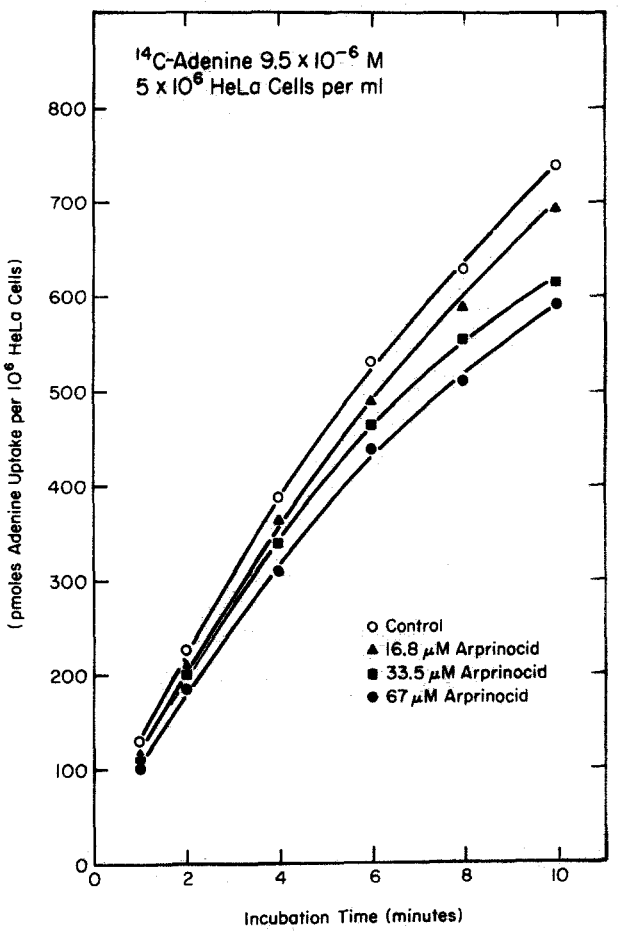


Fig. 4. Effects of arprinocid on adenine transport in HeLa cells. [^{14}C]Adenine (56 mCi/m-mole) was used as substrate.

temperature change apparently had very little effect on the partition coefficient.

DISCUSSION

The experimental data have provided evidence that arprinocid acts as a competitive inhibitor of hypoxanthine-guanine transport in HeLa cells. Its K_I values are lower than the K_m values of each of the two substrates. Should this inhibition be applicable to other cells and transport be the rate-limiting step in hypoxanthine-guanine salvage pathway as was indicated in Novikoff cells [5], arprinocid could be a genuine inhibitor of hypoxanthine-guanine salvage *in vivo*. It is also indicated by the kinetic studies that hypoxanthine and guanine share the same carrier-mediated transport system, whereas adenine is transported by a different carrier in HeLa cells. Data in Fig. 9 indicate that at lower concentrations of hypoxanthine (5×10^{-6} M), adenine becomes more inhibitory on its transport. This inhibition may not involve direct interaction with the hypoxanthine-guanine transport "carrier", which is in agreement with the previous observations on Chinese hamster lung fibroblasts [3] and Novikoff cells [5]. Arprinocid is relatively ineffective on adenine transport even though the drug molecule consists of an adenine moiety, whereas replacement of the adenine with hypoxanthine in the arprinocid molecule reduced the inhibitory effect on hypoxanthine transport (see Table 1). On the other hand, substitution at the 6-amino position of the molecule with alkyl groups enhanced the inhibition of hypoxanthine transport. All these structure–activity relationships may be related more closely to the affinity to the membrane carrier(s) than to the structure of the substrate with which the drug is competing. The differences between the inhibitory effects on hypoxanthine transport and anticoccidial activities as the alkyl substituents became bulkier could be reflections of differences in the hypoxanthine transport systems between HeLa cells and coccidia. But it also could result from the fact that the more hydrophobic derivatives of arprinocid tend to be associated more with the host cell membranes and become less available to the intracellular parasite, which would maintain the capability of blocking hypoxanthine transport in HeLa cells but reduce the anticoccidial activity. The lower activities of arprinocid-1-*N*-oxide in interfering with purine transport agree well with its lower anticoccidial activities in embryonated eggs [1]. Since both arprinocid and its 1-*N*-oxide were found in the urine of arprinocid-dosed chickens [15], it is not unlikely that both compounds are active agents against coccidia *in vivo* [1].

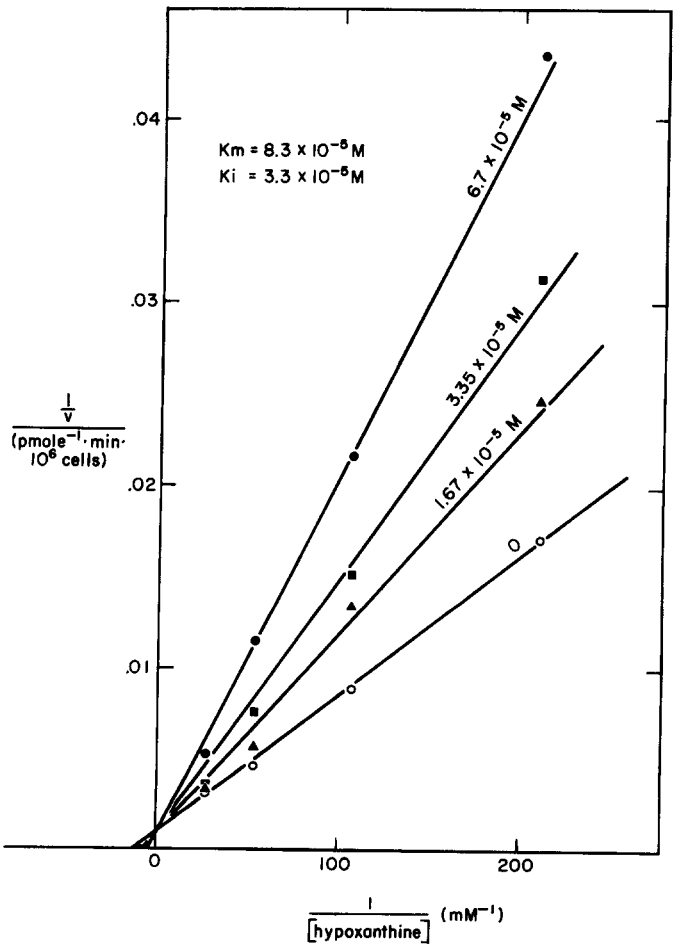


Fig. 5. Lineweaver–Burk plot of competitive inhibition of hypoxanthine transport in HeLa cells by arprinocid. See Fig. 2.

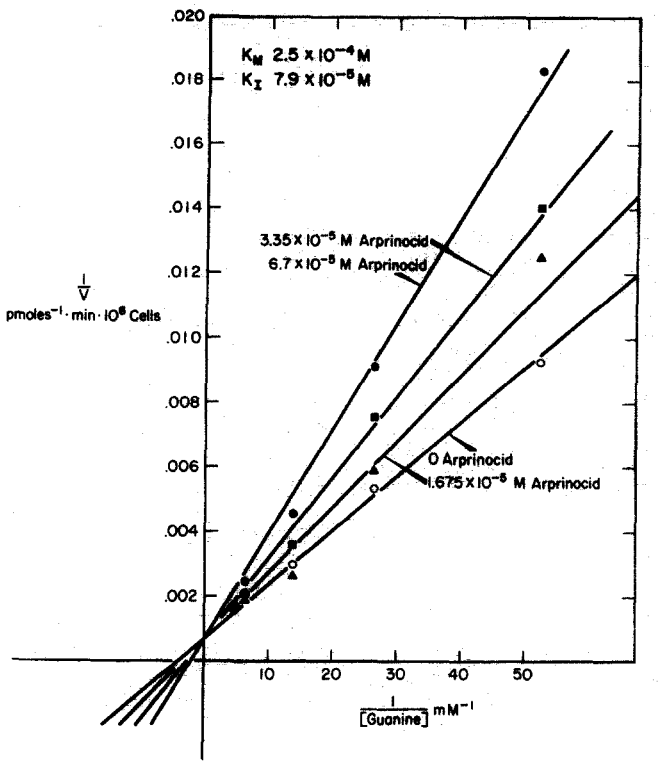


Fig. 6. Lineweaver–Burk plot of competitive inhibition of guanine transport in HeLa cells by arprinocid. See Fig. 3.

Table 1. Correlations between inhibition of hypoxanthine transport in HeLa cells and *in vivo* anticoccidial activities among arprinocid analogs

	% Inhibition of hypoxanthine uptake*		*In vivo* anticoccidial activities at 0.01%†	
	(20 μg drug/ml)	(10 μg drug/ml)	*E. acervulina*	*E. tenella*
I	49	31	A	A
II	11	10	I	I
III	33	17	I	I
IV	36	24	I	I
V		8	I	I
VI	50	30	I	I
VII	61	47	A	A
VIII	88	56	A	A
IX	61	43	A	A
X	56	39	A	A
XI	49	32	A	A
XII	54	35	A	A
XIII	93	86	I	I
XIV	95	91	A	I
XV	54	35	SA	A
XVI	46	28	MA	I
XVII	93	85	I	I
XVIII	42	27	MA	I
XIX	0	0	I	I
XX	23	13	I	I
XXI	47	33	MA	I
XXII	30	11	SA	I
XXIII	5	5	I	I

* [^{14}C]Hypoxanthine (53 mCi/m-mole) was present at 9.5 × 10^{-6} M. The rate of uptake was measured in pmoles hypoxanthine/min/10^6 HeLa cells during the first 6 min of incubation.

† Compounds were present in chicken's diet at 0.01% (w/w). A = active; MA = moderately active; SA = slightly active; and I = inactive.

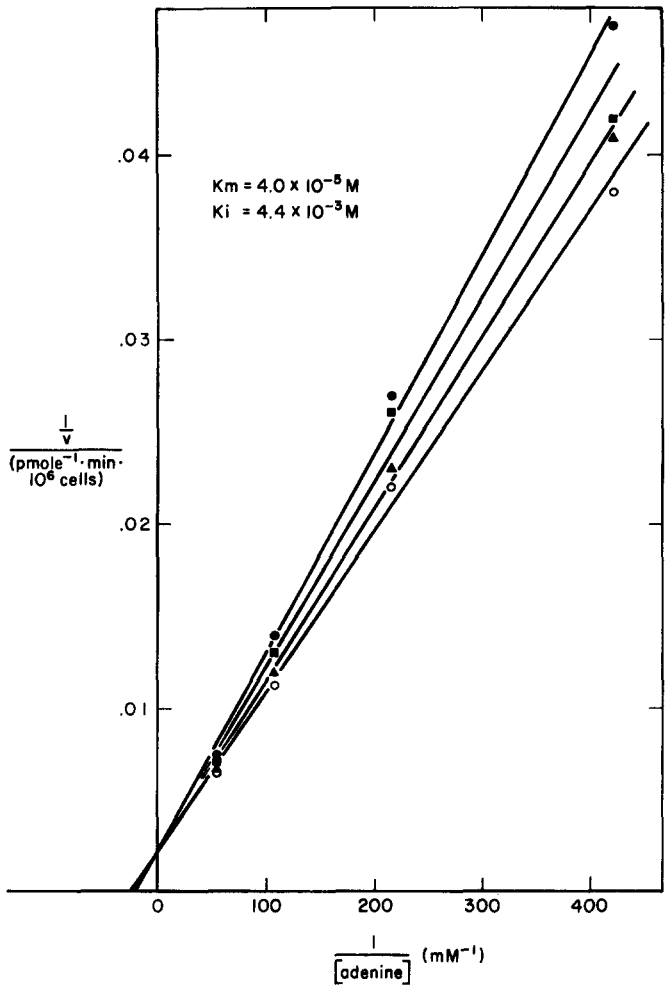


Fig. 7. Lineweaver–Burk plot of arprinocid effect on adenine transport in HeLa cells. See Fig. 4. Arprinocid concentrations: 0 (○——○); 1.675 × 10^{-5} M (▲——▲); 3.35 × 10^{-5} M (■——■); and 6.7 × 10^{-5} M (●——●).

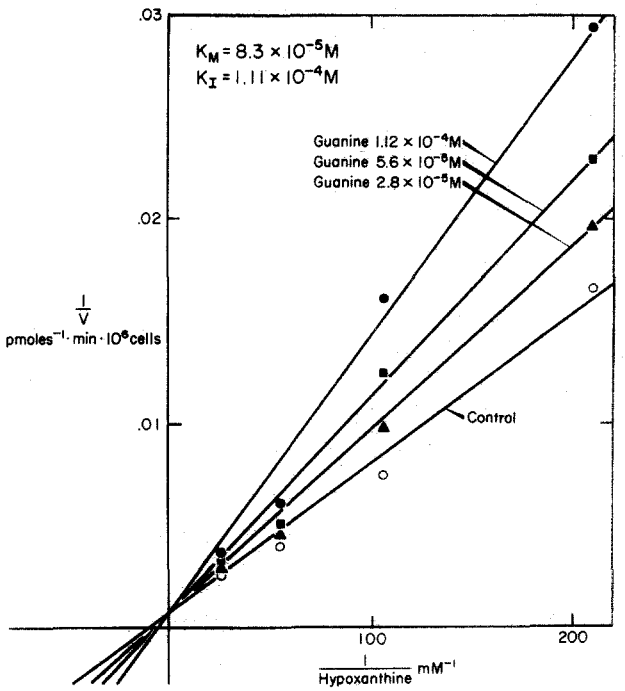


Fig. 8. Competitive inhibition of hypoxanthine transport in HeLa cells by guanine. [^{14}C]Hypoxanthine (53 mCi/m-mole) was used as substrate.

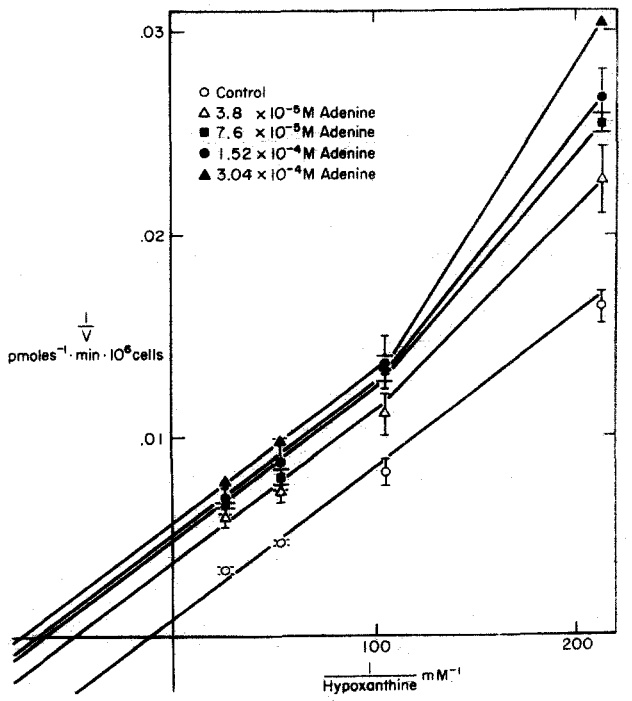


Fig. 9. Effects of adenine on hypoxanthine transport in HeLa cells. [^{14}C]Hypoxanthine (53 mCi/m-mole) was used as the substrate.

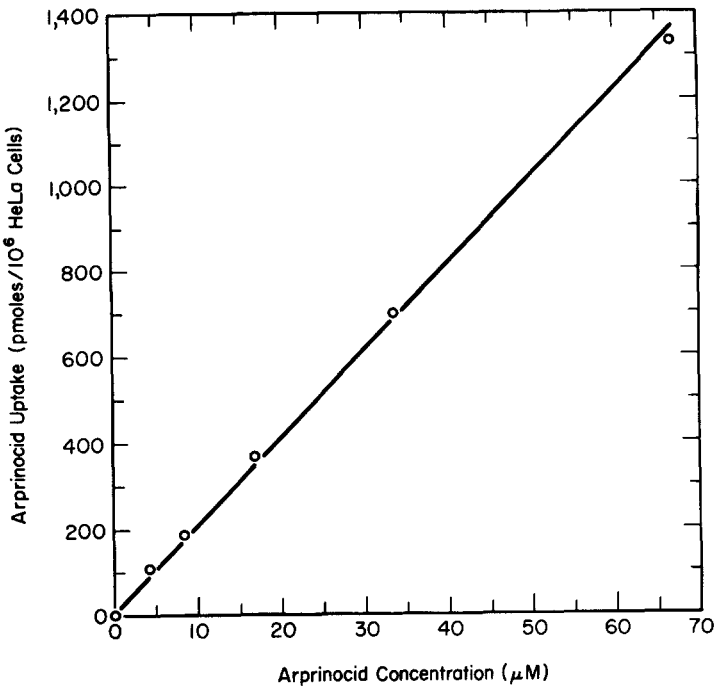


Fig. 10. Uptake of arprinocid by HeLa cells. [^{3}H]Methylene-arprinocid of 30.5 μCi/mg was used as substrate. Incubation was carried out at 37° for 5 min.

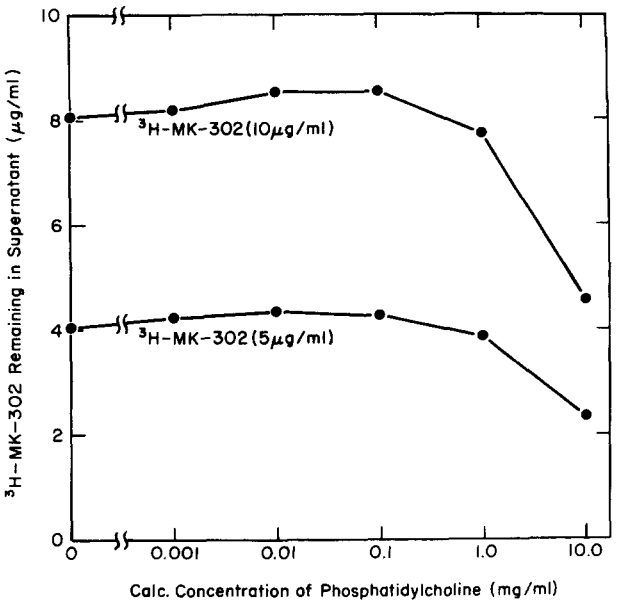


Fig. 11. Uptake of arprinocid by phosphatidylcholine liposomes. [^{3}H]Methylene-arprinocid at 30.5 μCi/mg was used as substrate. Incubation was carried out at 22° for 3.5 hr.

The correlation between inhibition of hypoxanthine transport in HeLa cells and *in vivo* anticoccidial activities among arprinocid analogs suggests that the mode of anticoccidial action of arprinocid is by inhibiting hypoxanthine transport in coccidia. This suggestion is supported by the previous observation that the drug inhibited incorporation of hypoxanthine into nucleic acids of *E. tenella* growing in cell cultures [1]. Other studies have demonstrated that *Toxoplasma gondii*, a close relative of *E. tenella*, can grow in Lesch–Nyhan mutant human fibroblasts and incorporate exogenous hypoxanthine directly into its nucleic acid [16], an indication that the coccidia may have a functioning hypoxanthine salvage pathway. Evidence also showed that *T. gondii* and *Plasmodium knowlesi* are incapable of purine *de novo* synthesis [1], and hypoxanthine is apparently the

only source of purine for *T. gondii* and *Plasmodium berghei* [1]. It is likely that the same holds true for the *Eimeria* family, and the inhibition of hypoxanthine transport will lead to inadequate purine supply for growth.

Direct quantitative study on the effect of arprinocid on hypoxanthine transport in *E. tenella* is technically not feasible at present because the parasite has active nucleotide turnover and nucleic acid synthesis only during its intracellular vegetative phase [13, 17]. It is very difficult to isolate *E. tenella* in that phase in a purified and viable form [18, 19].

The relatively low partition coefficient of [^{3}H]arprinocid to PC liposomes suggests relatively low affinity of arprinocid for the lipid bilayer structure. However, the drug was taken up by HeLa cells at an exceedingly high rate and to very high levels (Fig. 7). The failure to achieve saturation of drug uptake could mean that the experiments have been conducted at the low concentration position of a saturation curve due to the limited solubility of arprinocid. Calculations based on the phospholipid content of the HeLa cells [20] and on the data in Fig. 7 yielded an average partition coefficient of 686.5. This value, approximately 7-fold higher than that of PC liposomes, provides a clear indication of higher affinity of arprinocid to HeLa cells. This enhanced affinity could be attributable to the presence of hypoxanthine-guanine transport "carriers" in HeLa cell membranes. However, since 8×10^8 arprinocid molecules are "bound" per HeLa cell at 67 μM of the drug (Fig. 7) while 5×10^8 molecules are "bound" per *E. tenella* sporozoite at a 10 μM drug concentration (see Results), it will be difficult to assume the presence of hypoxanthine-guanine transport "carriers" at such high corresponding numbers in each cell. Most of the drug molecules are probably bound non-specifically, most likely to membrane proteins.

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