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MODE OF ANTICOCCIDIAL ACTION OF ARPRINOCID

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Abstract—An anticoccidial agent, arprinocid, 6-amino-9-(2-chloro-6-fluorobenzyl)purine, was found to selectively inhibit incorporation of hypoxanthine into nucleic acids of *Eimeria tenella* during its intracellular growth in cultured cells. Similar inhibitory effects were observed in HeLa cells, although preliminary data indicated that the drug had no apparent cytotoxicity to these cells. The drug also caused partial inhibition of hypoxanthine incorporation into the purine nucleotide pool, but stimulated formate incorporation into nucleic acids in HeLa cells. These results suggest that arprinocid does not inhibit *de novo* purine synthesis but blocks the hypoxanthine-guanine salvage pathway in HeLa cells. Similar effects exerted on coccidia could account for the mode of action of arprinocid because the parasites are believed to rely on hypoxanthine salvage as the main source of purines, whereas their hosts are capable of *de novo* purine synthesis.

Arprinocid, 6-amino-9-(2-chloro-6-fluorobenzyl)-purine, otherwise known as MK-302, is a newly discovered anticoccidial agent [1]. At dietary levels of 60–70 ppm, the drug exhibited anticoccidial activity in chickens heavily infected with various virulent species of coccidia [1, 2]. Oocysts of these intracellular sporozoan parasites, produced by the infected host under suboptimal dosages of the drug, showed reduced capability of sporulation and, consequently, decreased infectivity [1, 3–5]. The drug had a maximum tolerated diet concentration of about 95 ppm in chickens after 8-week pen trials [2]. However, it exhibited no apparent antibacterial, anthelmintic, antifungal or antitumor activity (B. M. Miller, Merck Institute, personal communication), nor did it show any effect on tobacco plants [1].

To understand the mode of anticoccidial action of arprinocid, one has to look for biological activities in coccidia which are unique and/or essential for the development of the parasite, but which are different and/or nonessential to bacteria, nematodes, fungi, plants or animals. The chemical structure of the drug, which is essentially an adenine molecule linked to a substituted benzyl group, suggests purine metabolism of the parasite as a possible target. Urine samples from chickens treated with labeled arprinocid contained only two major radioactive compounds, the original drug and arprinocid-1-*N*-oxide [6]. The same two compounds were also identified as the major products after incubation of arprinocid with chicken liver microsomes [7]. Indeed, it was indicated originally that arprinocid-1-*N*-oxide was active *in vivo* against coccidiosis [1]. Thus, either arprinocid or its 1-*N*-oxide, or both compounds, could be the true active compound against coccidia. 2-Chloro-6-fluorobenzyl alcohol and 2-chloro-6-fluorotoluene were inactive against coccidiosis in chickens (C. C. Wang, unpublished observations).

Some biochemical studies on derivatives of 9-benzyladenine have been done in the past. Baker and Sachdeu [8] observed inhibition of mammalian liver glutamic dehydrogenase by 9-(*p*-carboxybenzyl)-adenine with an ID_{50} value of 27 mM. The inhibition was competitive with NAD due to apparent similarity in conformation between the compound and the AMP moiety of NAD. 9-Benzyladenine inhibited *Escherichia coli* succinoadenylate kinosynthetase with an ID_{50} of 0.50 mM [9] and calf intestinal mucosa adenosine deaminase with an ID_{50} of 0.10 mM [10]. Baker [11] theorized that these enzymes may all have hydrophobic regions at their active sites which resulted in higher affinity to the adenine derivatives carrying a 9-position hydrophobic moiety.

The purpose of our investigation was to examine biochemical activities of arprinocid to elucidate its mechanism of anticoccidial action. This effort was aided by involving HeLa cells for quantitative studies and the use of a reference drug, mycophenolic acid [12]. The drug possesses antibacterial [13], antifungal [14] and anticancer activities [15], and has been identified as a potent inhibitor of IMP dehydrogenase [16, 17], thereby blocking GMP formation by both *de novo* synthesis and hypoxanthine salvage pathway [18]. However, it does not affect salvage of guanine and its conversion to GMP. Inclusion of this drug in our investigations has helped us in identifying the specific action of arprinocid on purine metabolism.

MATERIALS AND METHODS

Materials. A pure strain of *Eimeria tenella* (Merck No. 18) was maintained in the laboratory by monthly passages in chickens [19]. Unsporulated and sporulated oocysts and sporozoites of *E. tenella* were harvested and purified according to procedures described previously [19]. Crude extracts of unsporulated oocysts were prepared for enzyme assays by sonication and centrifugation at 100,000 *g* for 1 hr [20]. HeLa S3 cells grown in Eagle's minimal essential suspension medium (EMEM) supplemented with 10% fetal calf serum at 37° were obtained from the Roche Institute, Nutley, NJ.

Arprinocid, arprinocid-1-*N*-oxide, and [^{3}H]methylene-arprinocid (122 μCi/mg) were prepared in purified form (>99 per cent) at Merck & Co., Inc., Rahway, NJ. Mycophenolic acid was kindly provided by Eli Lilley &

Co., Indianapolis, IN. Labeled purines, pyrimidines and nucleosides were purchased from New England Nuclear, Boston, MA. A sample of purified *Bacillus subtilis* IMP dehydrogenase was a gift from Dr. L. Kaplan of the Merck Institute. All the other chemicals used in the studies were of the highest purity available from commercial sources.

Biological assays. The *in vivo* anticoccidial activity was tested in a standard assay in which 12-day-old White Leghorn chickens were inoculated *per os* with 5×10^4 *E. tenella* sporulated oocysts and maintained in wired cages. Bloody droppings, caecal lesions and oocyst counts were recorded 6 days post-inoculation to indicate the severity of infection [21]. All the drugs were administered via the diet. An alternative way of testing anticoccidial activity was by injecting *E. tenella* sporozoites and the test compounds into the allantoic cavity of 12-day-old White Leghorn embryonated eggs [22]. The massive hemorrhaging 4 days after inoculation, usually accompanied by death of the embryo, and the focal lesions formed on the chorioallantoic membrane were used as indicators of development of the parasite.

Enzyme assays. Dihydrofolate reductase activity was assayed as described previously [20]. Glucose-6-phosphate dehydrogenase activity was determined by a spectrophotometric assay [23]. IMP dehydrogenase was assayed by the method of Magasanik *et al.* [24]. Assays for hypoxanthine-guanine phosphoribosyl transferase were carried out according to the method of Schmidt *et al.* [25]. Cyclic AMP phosphodiesterase [26], xanthine oxidase [27], adenosine deaminase [28], nucleoside phosphorylase [29], glutamate dehydrogenase [30], malate dehydrogenase [31], isocitrate dehydrogenase [32] and fatty acid elongation enzyme [33, 34] were assayed according to the published procedures noted.

Autoradiography. Kidney epithelial cells of 19-day-old White Leghorn chick embryos were cultivated in EMEM plus 10% fetal calf serum at 37° for 3 days to form monolayers [35]. The cultures were inoculated with freshly purified sporozoites of *E. tenella*, and the incubation was continued for 3 more days in EMEM plus 1% dialyzed fetal calf serum to allow development of the parasite to second stage schizogony [35]. The infected cultures were then incubated in hypoxanthine-free Waymouth's medium (Grand Island Biological Co., Grand Island, NY) for 1 hr. Labeled substrate was added and the incubation was continued for 2 more hr. The monolayers were rinsed with balanced salt solution, fixed in neutral formalin at 37° for 30 min, washed in 80% ethanol, air dried, treated with 5% trichloroacetic acid (TCA) at 4° for 10 min and rinsed in tap water for 1 hr [36]. For labeling of DNA, the samples were treated with ribonuclease A at 0.2 mg/ml (Worthington Biochemicals, Freehold, NJ) in balanced salt solution at 37° for 30 min before the rinse in tap water [36]. The samples were then coated with 0.5% collodion, dried and dipped in Ilford G5 Nuclear tract emulsion diluted 1:1. After exposure at 4° under N_2 for 3–8 weeks, samples were developed in Kodak D-19 (diluted 1:2) at 18° for 4 min, rinsed and fixed in Kodak fixer for 1 min, rinsed in tap water, air dried and stained with hematoxylin-eosin.

Assay of radioactive substrate incorporation in HeLa cells. HeLa cells in suspension culture were harvested at the log phase of growth and resuspended in EMEM + 10% fetal calf serum to a final concentration of 2×10^5 cells/ml. Labeled substrates and drugs were added and the cell suspensions were incubated at 37°. Aliquots of 2.0 ml cell suspension were taken after various time intervals of incubation, mixed with 2.0 ml of ice-cold 10% TCA and incubated on ice for 30 min. Pellets were collected by 10,000 *g* centrifugation for 10 min, washed three times in ice-cold 5% TCA and dissolved in 0.5 N NaOH before the radioactivity was counted in a Packard Tri-Carb liquid scintillation spectrometer.

Radioactivity incorporated into the purine nucleotide pool was determined in the washed HeLa cells. The cells were extracted with cold 0.2 M perchloric acid, neutralized with 4 M KOH and centrifuged [18]. The supernatant fraction was mixed with nonradioactive purine nucleotides which were then separated by high voltage paper electrophoresis and located under u.v. light [37]. Labeled purine nucleotides were identified in a Packard radiochromatogram scanner model 7201. For higher resolution the paper tracks were cut in 0.5 cm segments. The nucleotides were extracted in small aliquots of water, and radioactivities were counted in a liquid scintillation spectrometer. Alternatively, the nucleotides were separated by PEI-cellulose thin-layer two-dimensional chromatography [38]. Nonradioactive samples were included to locate individual purine nucleotides which were scraped from the plates and counted.

RESULTS

Activities of arprinocid in biological assays. Medication of *E. tenella*-infected chickens with 60 ppm arprinocid in the diet arrested development of most of the parasites at their trophozoite stage. Hematoxylin-eosin stain of the caecal tissue from the birds indicated confinement of the trophozoites to the superficial caecal folds and basal caecal gland. No symptom of coccidiosis could be observed under the medication even when it was delayed up to 72 hr after infection. Adding adenine or adenosine to the diet up to 1000 ppm had no effect on the activity of arprinocid.

Injection of 250 μg arprinocid into the allantoic cavity of *E. tenella*-infected embryonated eggs fully controlled the development of the parasite. The injection could be delayed up to 72 hr without losing control. Simultaneous addition of 500 μg adenine or adenosine to the allantoic cavity showed no effect on the drug activity. By careful titration, the minimal effective dosage of arprinocid by allantoic cavity injection with a 72-hr delay was estimated to be 25 μg/egg, whereas that of arprinocid-1-*N*-oxide was 100 μg/egg. Since there is little metabolic activity in the allantoic cavity of an embryonic egg [39], interconversion between arprinocid and its 1-*N*-oxide would have been limited. The higher potency of arprinocid suggests that it is the active form against coccidia, and the rest of the studies have been based on this assumption.

Inhibition of E. tenella *dihydrofolate reductase by arprinocid.* The maximum solubility of arprinocid in aqueous solution containing 1% dimethylsulfoxide (DMSO) is about 67 μM at 37°. The drug was tested at

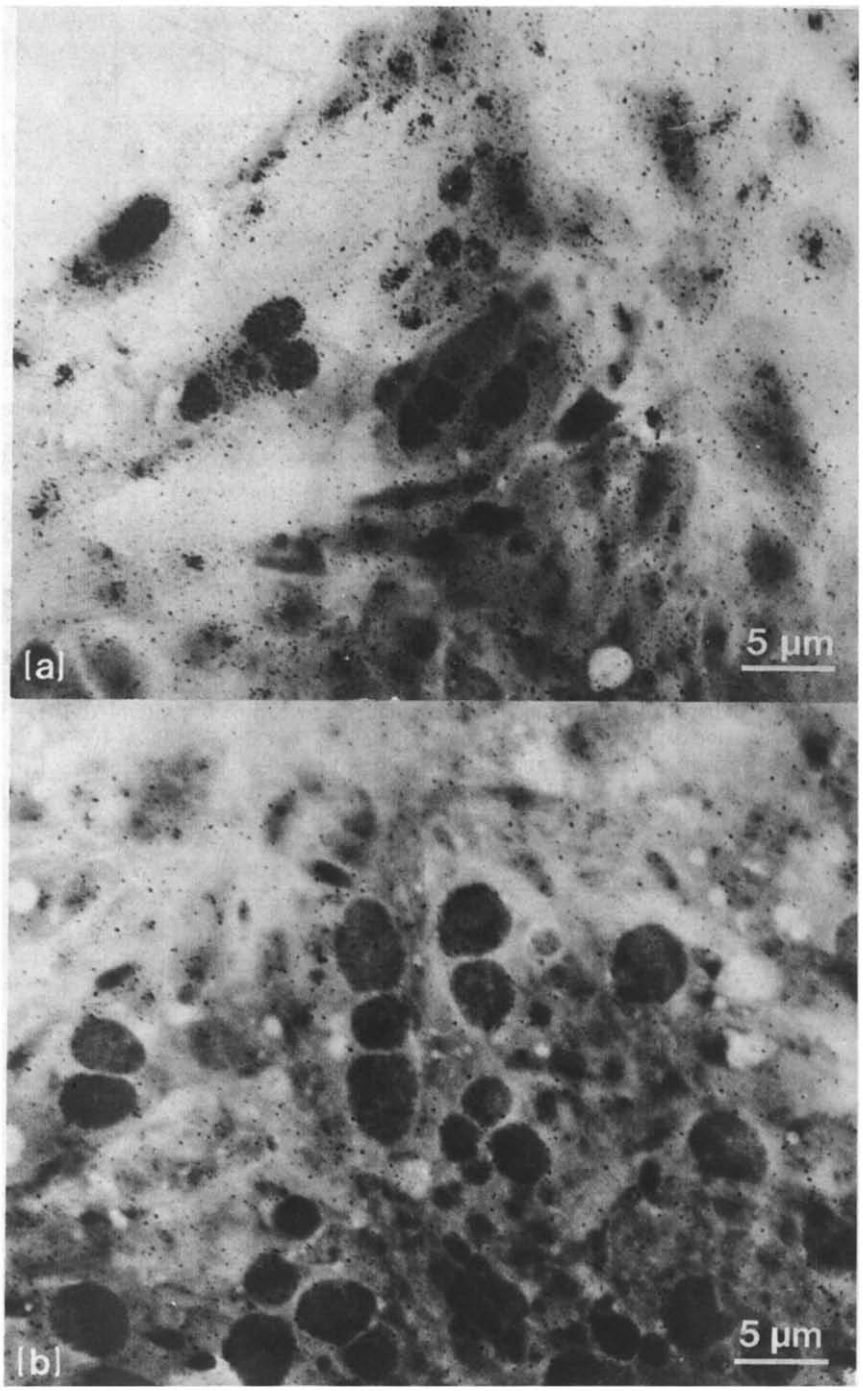

Fig. 1. Autoradiographic studies of the effect of arprinocid on [^{3}H]hypoxanthine incorporation into TCA-insoluble fraction in *Eimeria tenella* growing in cultured embryonic chick kidney epithelial cells. Panel a: pulse-labeling without the drug; panel b: pulse-labeling in the presence of 67 μM arprinocid.

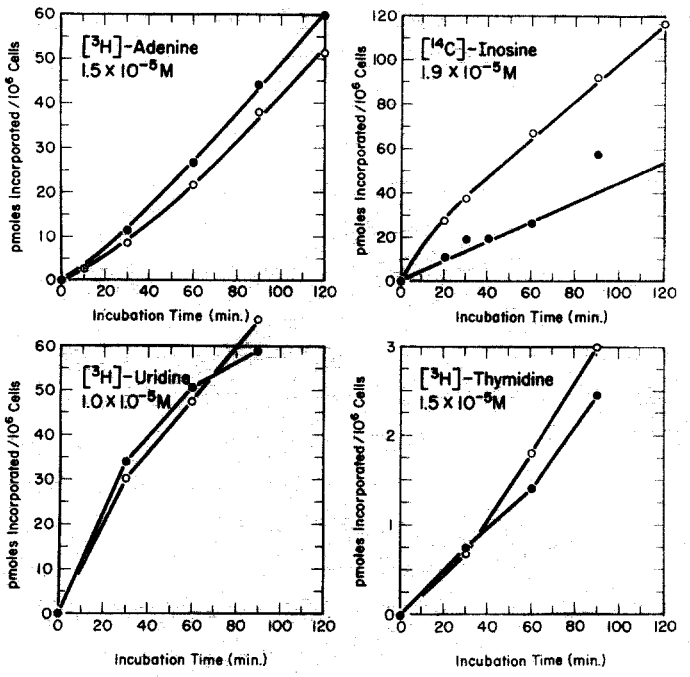

Fig. 2. Inhibition of substrate incorporation into TCA-insoluble fraction in HeLa cells by arprinocid. Specific activity: [³H]adenine, 15 Ci/m-mole; [¹⁴C]inosine, 52 mCi/m-mole; [³H]uridine 9.8 Ci/m-mole; and [³H]thymidine, 2.0 Ci/m-mole. Key: control (○——○); and 67 μM arprinocid (●——●).

this concentration in 1% DMSO against cow milk xanthine oxidase (Worthington), crude chicken liver hypoxanthine-guanine phosphoribosyl transferase, crude chicken liver adenosine deaminase, crude swine intestinal mucosal nucleoside phosphorylase and crude *E. tenella* cAMP phosphodiesterase (2.0 nmoles cAMP hydrolyzed/min/10^6 unsporulated oocysts), and no detectable effect was demonstrated. However, it did exert potent inhibition of *E. tenella* dihydrofolate reductase by competing with NADPH. Kinetic studies indicated a K_I value of 3.00×10^{-6} M, whereas similar studies on the enzyme from chicken liver [20] gave a higher K_I of 4.50×10^{-4} M.

Another enzyme identified in the crude extract of *E.*

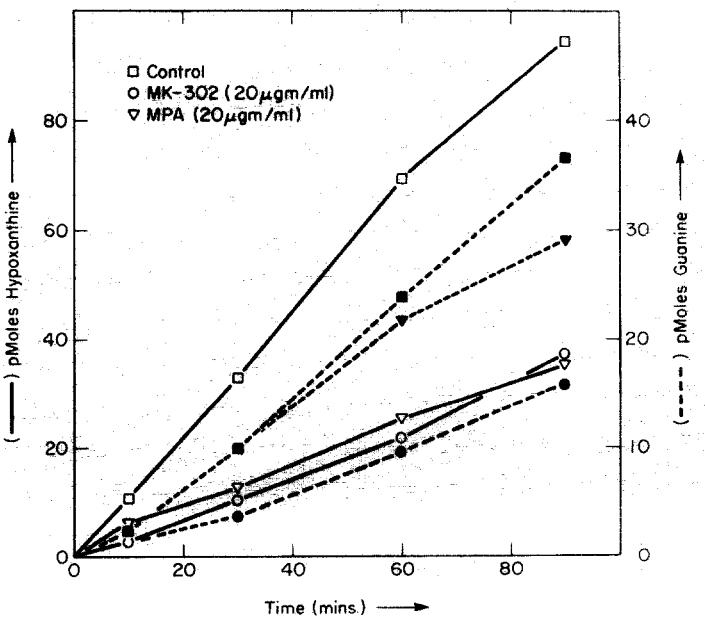

Fig. 3. Inhibition of incorporation of [¹⁴C]hypoxanthine and [³H]guanine into TCA-insoluble fraction of HeLa cells by arprinocid. HeLa cells were double-labeled with [¹⁴C]hypoxanthine (2.4×10^{-6} M, 53 mCi/m-mole) and [³H]guanine (2.2×10^{-6} M, 112 mCi/m-mole). Key: control (□, ■); 67 μM arprinocid (MK-302) (○, ●); and 61 μM mycophenolic acid (MPA) (▽, ▼).

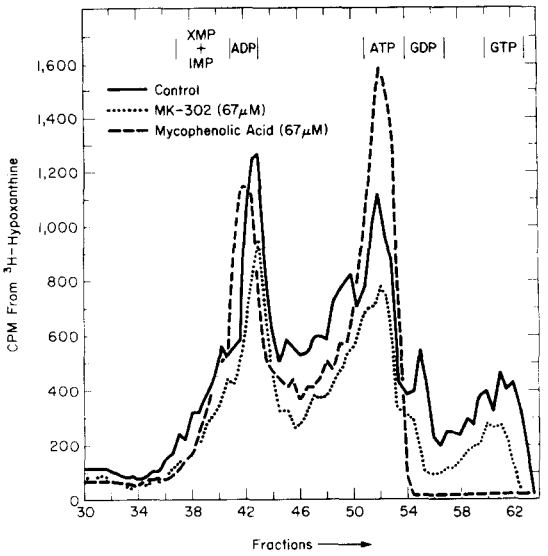

Fig. 4. Electrophoretic pattern of purine nucleotides labeled with [^{3}H]hypoxanthine in HeLa cells. Log-phase HeLa cells in EMEM plus 10% fetal calf serum at 10^7 cells/ml were pulse-labeled with [^{3}H]hypoxanthine (5×10^{-5} M, 0.25 Ci/m-mole) at 37° for 20 min. The extract (50 μl) of nucleotides from 5×10^5 HeLa cells was analyzed by high-voltage paper electrophoresis. For the total radioactivities, there were 308,000 cpm in the control, 250,000 cpm in the arprinocid-treated and 331,000 cpm in the mycophenolate-treated samples. There was little radioactivity in Fractions 1–30 and Fractions beyond 60.

tenella unsporulated oocysts, glucose-6-phosphate dehydrogenase (171 μmoles/min/mg of protein, K_M (NADP) = 1.50×10^{-5} M), was also inhibited by arprinocid. The inhibition was competitive with NADP and had an apparent K_I value of 8.98×10^{-5} M. The same enzyme from chicken liver had a K_I of 7.50×10^{-3} M for the drug. Other NADH–NADPH-dependent dehydrogenases found in the unsporulated oocysts of *E. tenella* were glutamate dehydrogenase (22.8 μmoles/min/mg of protein), isocitrate dehydrogenase (1.0 μmole/min/mg of protein), and fatty acid elongation enzyme (16 nmoles/min/mg of protein), and when tested with arprinocid showed no inhibitory effects.

Inhibition of hypoxanthine incorporation in E. tenella *by arprinocid.* It was considered possible that the preferential inhibition of *E. tenella* dihydrofolate reductase by arprinocid might be the mode of anticoccidial action. The inhibitor was tested by monitoring synthesis of DNA in the parasites to see if it would be blocked by the drug. Since *E. tenella* cannot incorporate exogenous thymidine into its DNA [36], pulse-labeling of DNA in *E. tenella* schizonts with [^{3}H]adenine (3.33×10^{-7} M, 5 μCi/ml) or [^{14}C]uracil (1.78×10^{-5} M, μCi/ml) was carried out [40], and monitored by autoradiography. The results showed similar densities of silver granules in the schizonts when the labeling took place both in the presence and absence of arprinocid (67 μM). However, the incorporation of [^{3}H]hypoxanthine (6.0×10^{-7} M, 5 μCi/ml) into DNA or the total nucleic acid fraction, illustrated in Fig. 1, was clearly inhibited by 67 μM arprinocid. This inhibition was found in both the parasite and the actively growing host cells. The drug itself, [^{3}H]methylene-arprinocid (6.7×10^{-5} M, 2.5 μCi/ml), labeled heavily both the parasite and the host cells but was not incorporated into TCA-insoluble fraction to any detectable extent when examined by autoradiography.

Inhibition of incorporation of hypoxanthine, guanine and inosine in HeLa cells by arprinocid. Since active nucleic acid metabolism in *E. tenella* takes place only during its intracellular phase [20], more quantitative information on the subject was difficult to obtain due to technical problems. A substituting model system thus became necessary for further studies. Quantitative assays were carried out on the incorporation of purines, pyrimidines and their nucleosides into TCA-insoluble fractions of the HeLa cells. The effects of arprinocid in this assay were investigated. The results, illustrated in Figs. 2 and 3, agree with the autoradiographic data on *E. tenella*. Incorporation of hypoxanthine, inosine and guanine was inhibited by the drug, whereas that of uridine, thymidine and adenine was not affected, suggesting no effect on nucleic acid synthesis *per se*. Because of the resemblance between *E. tenella* and HeLa in responding to arprinocid, the latter was chosen for more detailed studies. Data showed that 67 μM arprinocid inhibited incorporation of hypoxanthine by 64 per cent, inosine by 43 per cent and guanine by 58 per cent in HeLa cells after a 2-hr incubation.

Mycophenolic acid was included in the HeLa cell experiments as a reference drug. It had been tested on a purified sample of *B. subtilis* IMP dehydrogenase, and the kinetic data revealed it to be a competitive inhibitor of NADH with a K_I value of 3.2×10^{-6} M. It was also an uncompetitive inhibitor of IMP and showed a K_I value of 2.9×10^{-6} M (R. L. Stotish and C. C. Wang, unpublished observations). The drug and arprinocid were tested in double-labeling experiments illustrated in Fig. 3. Both compounds blocked the incorporation of hypoxanthine into the TCA-insoluble fraction but only arprinocid inhibited incorporation of guanine. Mycophenolic acid had no effect on guanine incorporation as would be expected from its mode of action. Further analyses indicated that guanine incorporation was also inhibited by hypoxanthine or inosine, and hypoxanthine incorporation was inhibited by guanine or inosine. The inhibitory effect of inosine on guanine incorporation has an ID_{50} value approximately equivalent to the concentration of guanine in the medium.

The effects of arprinocid and mycophenolic acid on incorporation of [^{3}H]hypoxanthine into the purine nucleotide pool of HeLa cells were examined. High voltage paper electrophoresis of samples after 20 min of pulse-labeling indicated significant inhibition of conversion of hypoxanthine to GDP and GTP by mycophenolic acid (Fig. 4). Arprinocid, however, showed no apparent effect on any specific nucleotide, but, rather had a general inhibitory effect on the incorporation of hypoxanthine into all the nucleotides, i.e. the labeled pool size became smaller (Fig. 4). PEI-cellulose thin-layer chromatography enabled separation among all the purine nucleotides and provided indications that incorporation of [^{3}H]hypoxanthine into GDP and GTP was

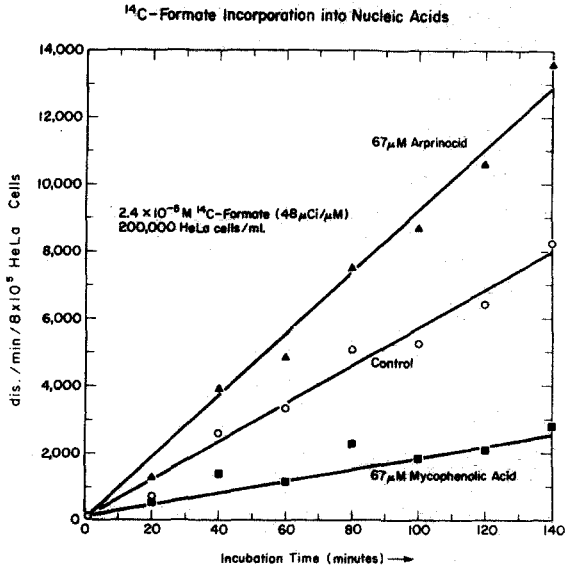

Fig. 5. [^{14}C]Formate incorporation into the nucleic acid fraction of HeLa cells. Duplicate samples were collected for measuring amounts of [^{14}C]formate incorporated into cold and hot 5% TCA-insoluble fractions. The hot 5% TCA-insoluble fractions, obtained after being heated at 90° for 10 min, amount to 3000 ± 1000 dis./min/8×10^5 HeLa cells in all the experiments after incubation for 140 min. The difference between the two was taken as the amount of [^{14}C]formate incorporated into nucleic acids.

inhibited by 67 μM mycophenolic acid, whereas arprinocid at the same concentration only partially reduced the radioactivity in IMP.

Stimulation of formate incorporation in HeLa cells by arprinocid. HeLa cells were pulse-labeled with [^{14}C]formate and the radioactivity incorporated into cold TCA-insoluble and hot TCA-insoluble fractions among duplicated time samples was measured. The cold TCA-insoluble fraction is considered to contain both nucleic acids and proteins, whereas the hot TCA-insoluble fraction consists of only proteins. These experiments were designed to see if arprinocid had any effect on the incorporation of formate into newly synthesized purines which, in turn, become incorporated into nucleic acids [41]. Figure 5 shows the time course of the incorporation of formate into nucleic acids which was inhibited by mycophenolic acid but stimulated by arprinocid. The incorporation of [^{14}C]formate into hot TCA-insoluble fractions was unchanged by the presence of either drug.

Lack of effect of arprinocid on growth of HeLa cells. Cultivation of HeLa cells as monolayers in the presence of arprinocid or mycophenolic acid was tested. Figure 6 demonstrates that mycophenolic acid reduced the number of cells after 24 hr of incubation; the inhibitory effect could be partially reversed by adding guanosine or guanine but not by adding adenine to the incubation medium. Arprinocid at 67 μM caused partial reduction in cell numbers which was not affected by the presence of guanosine, guanine or adenine.

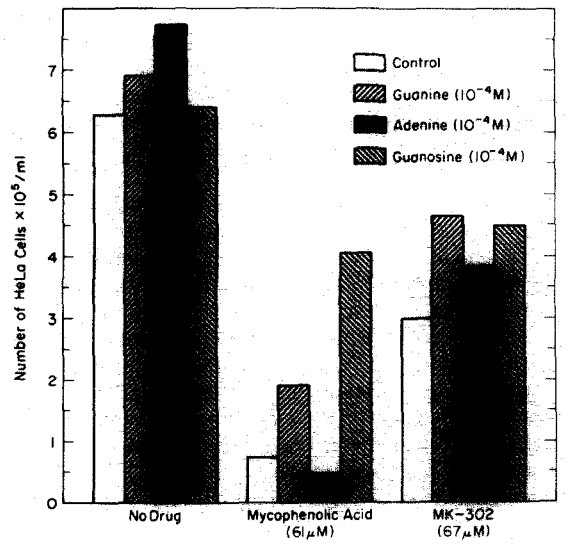

Fig. 6. Effects of mycophenolic acid and arprinocid on cultivation of HeLa cells. HeLa cells were cultivated in EMEM, 10% fetal calf serum plus drugs and other substrates under 5% CO_2 at an initial concentration of 2×10^5 cells/ml. The cells were examined and counted microscopically after 24 hr of incubation at 37°.

DISCUSSION

Our studies indicate that aprinocid is capable of arresting development of the parasite at any intracellular stage. It seems that the drug blocks some aspects of metabolism in the parasite which may be essential throughout its intracellular growth. The failure in reversing that activity with adenine or adenosine suggests lack of direct competition between the drug and the substrates.

Arprinocid acted like 9-(*p*-carboxybenzyl)adenine [8] in competing with NADPH which resulted in inhibition of dihydrofolate reductase and glucose-6-phosphate dehydrogenase. It may be interesting to note that dihydrofolate reductase was among the enzymes postulated by Baker [11] to possess hydrophobic regions at the active sites. The arprinocid inhibition of *E. tenella* dihydrofolate reductase *in vitro*, though having a 100-fold lower K_I than that of chicken liver, was not, however, reflected in reduction of DNA synthesis in the parasite. Since *E. tenella* is incapable of incorporating exogenous thymidine into DNA [36], the lack of arprinocid inhibition of DNA synthesis must mean adequate functioning of dihydrofolate reductase *in vivo* under the influence of the drug. The *in vitro* inhibition of *E. tenella* glucose-6-phosphate dehydrogenase by arprinocid is unlikely to be the mode of action of the drug not only because of its weaker inhibitory effect but also because carbohydrate utilization of coccidia is mainly via glycolysis [42].

The arprinocid inhibition of incorporation of hypoxanthine into nucleic acids of *E. tenella* schizonts, also noted in embryonic chick kidney epithelial and HeLa cells, could be the mode of anticoccidial action of the drug. This suggestion is based on the rationale that, while chickens possess high activities in purine *de novo* synthesis in the liver [43], the parasite may have to depend on exogenous purines. Ample evidence has indicated this to be true among intracellular protozoan parasites. It was noted that neither formate nor glycine

was incorporated into the nucleic acids of *Toxoplasma gondii*, a close relative of *E. tenella*, suggesting lack of *de novo* purine synthesis [44]. When *T. gondii* was cultivated in Lesch–Nyhan mutant human fibroblasts, exogenous hypoxanthine was incorporated into the parasite, indicating functioning hypoxanthine phosphoribosyl transferase [45]. Similarly, there is a lack of incorporation of formate into the purines of cultured *Plasmodium knowlesi* [46]. Manandhar and Van Dyke [47] incubated erythrocyte-free *Plasmodium berghei* with [^{3}H]adenosine and found the latter deaminated to inosine and then deribosylated to hypoxanthine on the cellular surface of the parasite before entering the cell. A similar process was observed in *T. gondii* (E. R. Pfefferkorn, personal communication).

We have recently detected in the crude extracts of *E. tenella* unsporulated oocysts [19] substantial hypoxanthine phosphoribosyl transferase activities (766 pmoles/min/mg of protein), but little glutamine-5-phosphoribosyl-1-pyrophosphate (PRPP) amidotransferase (<1 pmole/min/mg of protein) which is known to regulate *de novo* purine synthesis [48] (unpublished observations). This information tends to support the idea that exogenous hypoxanthine constitutes the main source, if not the only source, of purines for *E. tenella*.

More analysis of the effects of arprinocid on HeLa cells indicates inhibition of incorporation of hypoxanthine and guanine into nucleic acids. The inhibition of inosine incorporation was probably due to conversion of inosine to hypoxanthine by purine nucleoside phosphorylase prior to its transport into HeLa cells, which has been demonstrated as the mechanism of inosine transport in mouse L cell membrane vesicles [49], and as one of the two inosine transport systems in human fibroblasts [50]. However, it is also possible that arprinocid directly inhibits inosine transport, and that inosine, hypoxanthine and guanine compete for a common site on the cell surface for transport. The "site" may be blocked by arprinocid. The effects of arprinocid were also reflected in the purine nucleotide pool by partial reduction of hypoxanthine incorporation into IMP and other purine nucleotides. These data coupled with the noted stimulation of formate incorporation into nucleic acids by arprinocid could be explained by a blockade of the hypoxanthine-guanine salvage pathway by the drug. The apparent stimulatory effect on *de novo* synthesis of purines could be a consequence of inhibition of purine salvage. There was about 45 μM hypoxanthine equivalent in fetal calf serum when one compared the serum with the dialyzed serum sample in HeLa cell [^{3}H]hypoxanthine transport assays [51] (unpublished results). The decreased phosphoribosylation of hypoxanthine and guanine results in accumulation of PRPP in HeLa cells which will, in turn, stimulate *de novo* synthesis of purines [52]. HeLa cells can grow by means of purine *de novo* synthesis without a functioning purine salvage which may explain the lack of a growth-inhibiting effect by arprinocid as indicated in Fig. 6. Thus, it is concluded that arprinocid inhibits the hypoxanthine-guanine salvage pathway in HeLa cells. Studies presented in the next communication [51] indicate that inhibition is by competitively interfering with the "carrier" mediated hypoxanthine-guanine transport system. This inhibition, reflected in *E. tenella* by arprinocid inhibition of hypoxanthine incorporation (Fig. 1), could be the mode of anticoccidial action of arprinocid.

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