ABSTRACT  Crude extracts of the oocysts of *Eimeria tenella*, a protozoan parasite of the coccidium family that develops inside the caecal epithelial cells of infected chickens, do not incorporate hypoxanthine or formate into purine nucleotides; this suggests lack of capability for *de novo* purine synthesis by the parasite. The extracts, however, contain high levels of activity of the purine salvage enzymes: hypoxanthine, guanine, xanthine, and adenine phosphoribosyltransferases and adenosine kinase. The absence of AMP deaminase from the parasite indicates that *E. tenella* cannot convert AMP to GMP; the latter thus has to be supplied by the hypoxanthine, xanthine, or guanine phosphoribosyltransferase of the parasite. These three activities are associated with one enzyme (HXGPTase), which has been purified to near homogeneity in high yield (71–80%) in a single step by GMP-agarose affinity column chromatography. The size of the enzyme subunit is estimated to be 23,000 daltons by NaDodSO₄ gel electrophoresis. Kinetic studies suggest differences in purine substrate specificity between *E. tenella* HXGPTase and chicken liver HGPRTase. Allopurinol preferentially inhibits the parasite enzyme by competing with hypoxanthine; a *K*ₐₜ = 22 μM.

All parasitic protozoa examined to date appear to be unable to synthesize the purine ring *de novo*, as reflected by the failure of radiolabeled glycine and formate to label nucleic acid purines of the parasites in minimal defined media. Of particular note are studies on *Trypanosoma cruzi* (1), *Leishmania brasiliensis* (2), *Plasmodium lophurae* (3), and *Trypanosoma megna* (4), which provide well-documented cases for lack of capability for *de novo* purine synthesis and, hence, dependence on purine salvage. Host hypoxanthine, adenine, and adenosine have been suggested as the three major sources of purines for these parasites (5), but recent evidence favors the hypothesis that hypoxanthine may be the main, if not the only, supply of purines to *Plasmodia* (6, 7), *Leishmania* (2, 8), and *Crithidia fasciculata* (8). Adenosine and adenine are probably converted to hypoxanthine by the parasite nucleoside hydrolase (9) and adenine aminohydrolase (2) of the parasites before incorporation into the nucleotide pool.

Among members of the coccidial family, a group of parasitic protozoa developing inside intestinal epithelial or muscle cells of infected animals, *Toxoplasma gondii* trophozoites were found incapable of incorporating glycine or formate into their DNA (10). They grow normally inside cultured Lesh–Nyhan skin fibroblasts, which lack hypoxanthine-guanine phosphoribosyltransferase (HGPRTase), and effectively incorporate exogenous hypoxanthine and guanine into nucleic acids (11). *T. gondii* has no detectable adenine phosphoribosyltransferase (APRTase) but has a high level of adenosine kinase (12). The latter activity is largely lost in a mutant resistant to 1-β-D-arabinofuranosyl-adenine (AraA). But wild-type and mutant *T. gondii* grown in cell culture are equally efficiently labeled by [3H]adenosine (12), suggesting that adenosine kinase does not play a major role in salvaging purines for the parasite. One other species of coccidia *Eimeria tenella*, which develops inside the caecal epithelial cells of chickens, effectively takes up hypoxanthine and preferentially incorporates label from it into nucleic acids when grown in cell culture (13, 14). Purine metabolism in this parasite is particularly interesting in view of the uricotelic metabolism of chickens and the high levels of hypoxanthine known to accumulate in avian tissues (15).

In this study, we have demonstrated that *E. tenella* cannot perform *de novo* purine synthesis and examined the detailed mechanism of purine salvage. A purine phosphoribosyltransferase that uses hypoxanthine and guanine as well as xanthine as substrates (HXGPTase) was identified in the parasite. The enzyme was purified and characterized.

Allopurinol, an analog of hypoxanthine innocuous for mammalian cells, is effective in vitro against *Leishmania* (16), *T. cruzi* (17), and African trypanosomes (18) through sequential conversion to allopurinol ribonucleotide and aminopurinol ribonucleoside mono-, di-, and triphosphates and eventual incorporation into the DNA of these parasites. It serves as a substrate for the HGPRTase of *Leishmania* destined for uricotelic metabolism, having *K*ₐₜ 68 times that of hypoxanthine (19). In this study, allopurinol was tested on *E. tenella* HXGPTase and chicken liver HGPRTase and found to be a preferential inhibitor of the parasite enzyme.

MATERIALS AND METHODS

Materials. Unsporulated oocysts of *E. tenella* strain 18 were propagated in chickens and harvested and purified as described (20). Radiolabeled substrates were from New England Nuclear or Research Products International. GMP-agarose [4% (w/v) agarose-NG-(CH₂)₉-NG-S-C-guanosine-5'-phosphate] was from P-L Biochemicals. 5-Phosphoribosyl-1-pyrophosphate (PRPP) was from Boehringer Mannheim, and 5-aminomidazole-4-carboxamide ribonucleotide (AICAR) and allopurinol were from Sigma. 5,10-Methylenetetr ah ydrofolate was prepared from tetrahydrofolate (Calbiochem) by a known procedure (21).

Enzyme Assays. Purine phosphoribosyltransferase activities were assayed by a modified procedure of Schmidt et al. (22). Two hundred microliters of 0.10 M Tris·HCl, pH 7.8/7 mM MgCl₂/1.0 mM PRPP containing bovine serum albumin at 50 μg/ml and 4.0 μM [8-¹⁴C]hypoxanthine (52.8 mCi/mmol; 1 Ci = 3.7 × 1₀¹⁶ becquerels), [2-¹⁴C]xanthine (48.0 mCi/mmol), [8-¹⁴C]guanine (54.7 mCi/mmol), or [8-¹⁴C]adenine (43.9 mCi/mmol) was incubated at 37°C, and reaction was initiated by adding enzyme. After 10 min of incubation, the reaction was terminated by the addition of an equal volume of an ice-cold 2 mM unlabeled purine base. The duration of incubation and substrate concentrations were varied in kinetic studies. Aliquots of the cooled reaction mixture were filtered through a glass-fiber filter

Abbreviations: H-, X-, G-, and APRTase, hypoxanthine, xanthine, guanine, and adenine, respectively (and combinations thereof), phosphoribosyltransferase, PPRP, 5-phosphoribosyl-1-pyrophosphate, AICAR, 5-aminimidazole-4-carboxamide ribonucleotide.

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evenly coated with 32 mg of polyethyleneimine-cellulose (Merk) preequilibrated with 1.0 mM NH₄OAc, pH 5.0. The loaded filter was washed with three 5-ml portions of the same solution and then soaked in Aquasol 2 (New England Nuclear). Levels of radioactivity were determined with a Beckman LS 8000 liquid scintillation spectrometer.

Purine ribonucleoside kinase and phosphotransferase activities were assayed by methods similar to those of Nelson et al. (23) using 1.3 mM [8-14C]inosine (5.2 mCi/mmol), [2-14C]-xanthosine (5.0 mCi/mmol), [8-14C]guanosine (5.3 mCi/mmol), or [8-14C]adenosine (5.5 mCi/mmol) as substrate. Reactions were terminated by filtering the mixtures through polyethyleneimine-cellulose filters, and radioactivities were assayed as described.

Adenosine deaminase was assayed according to Hoagland and Fisher (24). AMP deaminase activity was assayed by the method of Ashby and Frieden (25); the reaction was followed at 265 nm in a Beckman ACTA III spectrophotometer. Protein concentrations were determined by the method of Bradford (26) unless otherwise stated.

High-Pressure Liquid Chromatography. Purine bases, nucleosides, and nucleotides were separated and identified by paired-ion reverse-phase chromatography as described by Rowe et al. (27). A Partisil-10 ODS-2 (Reeve Angel) column (25 cm × 4.6 mm) equilibrated with 5.0 mM tetrabutylammonium hydroxide, adjusted to pH 6.0 with phosphoric acid/10% (vol/vol) methanol was washed with the same buffer for 10 min at a flow rate of 1.0 ml/min following sample injection. Then, concentration of methanol was increased linearly to 50% (vol/vol) over 15 min at the same flow rate and maintained at 50% for another 15 min. The eluate was collected in 0.5-ml fractions, and the radioactivity was determined as described above.

Affinity Column Chromatography. A supernatant fraction of the crude extract of E. tenella unsporulated oocysts was adjusted to 0.10 M Tris-HCl, pH 7.0/0.10 M MgCl₂/0.50 M KCl (TMK), and a 15.6-ml aliquot containing 86.3 mg of protein was loaded onto a 3 ml GMP-agarose column previously equilibrated with TMK buffer at 0–4°C. The loaded column was eluted with 250 ml of TMK buffer at a flow rate of 30 ml/hr and then with 40 ml of TMK buffer/10 mM PRPP at 10 ml/hr. The effluent was collected in 5-ml fractions and assayed for enzyme activities and protein content at 380 nm.

NaDodSO₄/Polyacrylamide Gel Electrophoresis. The procedure is that of Laemmli using 1 cm of 30% stacking gel and 10 cm of 12.5% running gel (28). Coomassie brilliant blue staining has the sensitivity to detect 0.05 µg of protein in the gel. Protein standards were from Boehringer Mannheim.

RESULTS

Lack of de Nocu Purine Synthesis in Extracts of E. tenella Oocysts. Freshly purified unsporulated oocysts of E. tenella were broken at 0–4°C by a brief sonication in 50 mM Tris-HCl, pH 7.0, at a density of 1.2 × 10⁹ oocysts per ml. The crude homogenate was centrifuged at 10,000 × g for 30 min, and the supernatant was collected and centrifuged at 100,000 × g for 1 hr. The supernatant, containing 5.4 to 5.5 mg of protein/ml, was immediately assayed for de novo purine synthesis as described by Rowe et al. (27). Freshly prepared cell-free extracts (25.5 mg of protein per ml) of livers obtained from 2-week-old White Rock chickens fasted for 24 hr before sacrifice were assayed in the same way. The results indicate no appreciable incorporation of radiolabel from either [14C]glycine or [14C]formate into the polyethyleneimine-cellulose-adsorbable fraction by crude extracts of E. tenella (Table 1). Chicken liver extracts catalyze both PRPP-dependent incorporation of [14C]glycine and AICAR-stimulated incorporation of [14C]formate into the probable purine nucleotide fraction. Under optimal conditions, ~28% of the total [14C]glycine and 9% of the total [14C]formate are incorporated into the nucleotide fraction after 20 min of incubation with chicken liver extract.

To verify that the assays reflected de novo purine synthesis, 2.0-m1 aliquots of the reaction mixtures were mixed with 0.1 ml of 4.2 M perchloric acid after the incubation. The samples were then chilled in an ice bath for 30 min, neutralized with 0.1 ml of 4.42 M KOH, centrifuged briefly, and concentrated to 0.1 vol by lyophilization. Aliquots (10 µl) of each extract were analyzed by high-pressure liquid chromatography. The results show that both [14C]glycine and [14C]formate remain largely unchanged after incubation with E. tenella extracts but chicken liver extracts convert some of the label in [14C]glycine to a major fraction that has a retention time corresponding to the intermediate ribonucleotides in de novo purine synthesis (27) and a smaller fraction corresponding to IMP (Fig. 1). [14C]Formate was partly incorporated into IMP, with much less into the intermediate ribonucleotide formyl AICAR, the immediate precursor to IMP.

Thus, this incubation system serves as a specific assay for de novo purine synthesis. The lack of incorporation of glycine or formate into purine nucleotides in extracts of E. tenella unsporulated oocysts indicates that these extracts are incapable of de novo purine synthesis.

Purine Salvage Enzymes in Extracts of E. tenella Oocysts. The extracts of E. tenella oocysts and chicken livers were also assayed for HPRTase activity. The results (Table 1) show a HPRTase specific activity in E. tenella 9 to 10 times that in chicken liver. When the assay mixtures were extracted with perchloric acid, and the contents were analyzed by HPLC, most of the label in [14C]hypoxanthine was incorporated by the E. tenella crude extract into a fraction corresponding to IMP (Fig. 2A). Similar results were obtained from the chicken liver extract except that less label was found in the IMP fraction (Fig. 2B).

The observation of the lack of de novo purine synthesis and the high HPRTase activity in E. tenella was followed up by comparison of the profile of purine salvage enzymes in E. tenella oocysts with that in chicken liver. The results showed significant

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Reaction mixture</th>
<th>Chicken liver</th>
<th>E. tenella</th>
</tr>
</thead>
<tbody>
<tr>
<td>[14C]Glycine</td>
<td>Complete</td>
<td>333</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>Lacking PRPP</td>
<td>7.7</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Containing boiled extract</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>[14C]Formate</td>
<td>Complete</td>
<td>139</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Containing AICAR</td>
<td>365</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Containing boiled extract</td>
<td>0</td>
<td>1.7</td>
</tr>
<tr>
<td>[14C]Hypoxanthine</td>
<td>Complete</td>
<td>44</td>
<td>402</td>
</tr>
<tr>
<td></td>
<td>Lacking-PRPP</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Each assay was carried out in 2.0 ml of a mixture of 4.0 µmol of L-aspartate, 4.0 µmol of L-glutamine, 5.0 µmol of ATP, 10 µmol of MgCl₂, 20.0 µmol of KCl, 100 µmol each of KCl and Tris-HCl, pH 7.8, 3.0 µmol each of PRPP and 5,10-methylenetetrahydrofolate, 20 µmol of phosphoenolpyruvate, 28.6 units of pyruvate kinase, cell-free extracts containing 5.0 mg of protein, and 4.0 µmol of AICAR when specified (27). After addition of 1.5 µmol of [14C]glycine (0.83 mCi/µmol), 4.0 µmol of [14C]formate (0.25 mCi/µmol), or 3.3 µmol of [14C]hypoxanthine (52.8 mCi/µmol), incubation was carried out at 37°C for 20 min. Purine nucleotides in 50 µl of incubation mixture were trapped on PEI-cellulose filters and washed, and radioactivity was assayed.
levels of HPRTase, APRTase, GPRTase, XPRPase, and adenosine kinase activities in *E. tenella* (Table 2). The products of all enzyme reactions were identified as the corresponding ribonucleotides by HPLC analysis (data not shown). There was no detectable inosine kinase, xanthosine kinase, guanosine kinase, purine nucleoside phosphoribosyltransferase, adenosine deaminase, or AMP deaminase activity in the parasite. The absence of the last two enzymes strongly suggests that AMP is not converted to GMP in *E. tenella*.

The chicken liver extract has a similar profile of purine salvage enzymes with lower specific activities, except that it has very low XPRPase activity but significant amounts of adenosine deaminase and AMP deaminase. The chickens are apparently capable of converting AMP to GMP.

Characterization and Purification of XPRTase from *E. tenella*. The profile of purine metabolism enzymes in *E. tenella* suggests that the only way for the parasite to make GMP is by its HPRTase, XPRTase, or GPRTase activity.

To determine whether the three purine phosphoribosyltransferase activities of *E. tenella* are in one, two, or three individual enzymes, heat inactivation experiments were carried out. The crude extract of *E. tenella* unsporulated oocysts was incubated at 37°C, and samples were taken after different periods of incubation time for assays of various transferase activities. The results show that, although APRTase activity remained relatively constant during the 3-hr incubation, HPRTase, XPRPase, and GPRTase were all inactivated with a similar time course, suggesting that these three activities reflect one enzyme (Fig. 3). Supporting evidence was obtained from a study of the optimal pH values for different enzyme activities. APRTase has a rather broad pH optimum and retains full activity at pH 9.0, whereas the other three activities share a bell-shaped curve in which the optimal pH is at 7.0–7.5, total inactivation is at pH 5.4, and 50% inactivation occurs at pH 9.0.

The enzyme, designated hypoxanthine-xanthine-guanine phosphoribosyltransferase (HXGPRTase), was purified by CMP-agarose affinity column chromatography. The elution profile indicates that most of the proteins pass through the column in TMK buffer (Fig. 4). Most of the HPRTase, XPRPase, and GPRTase activities were recovered in a sharp peak after addition of PRPP to the elution buffer. The total HXGPRTase activity (fractions 50–58) represents 71–80% yield. These fractions were pooled, concentrated to 1.0 ml by using a Millipore immersible CX unit, and dialyzed against 1 lter of 2.0 mM sodium phosphate buffer, pH 7.2, at 0–4°C with three changes of buffer in 16 hr. The dialyzed sample was lyophilized and dissolved in 40 μl of water. The protein content of this sample was determined by the fluorescamine spectrofluorometry method (29) and found to be 100 μg, which leads to an estimated purification of HXGPRTase of 610 to 688-fold.

The same concentrated sample was examined by NaDodSO4/polyacrylamide gel electrophoresis, and only a single protein band was found in the sample (Fig. 5). No other protein bands are visible when the gel is overloaded with 40 μg of protein. It is thus concluded that, assuming that the protein band rep-

![Fig. 1](image1.png)

**FIG. 1.** High-pressure liquid chromatography analysis after incubation of crude extracts with labeled substrates. (A) *E. tenella* oocysts and [14C]formate. (C) *E. tenella* oocysts, [14C]formate, and AICAR. (D) Chicken livers, [14C]formate, and AICAR.

![Fig. 2](image2.png)

**FIG. 2.** High-pressure liquid chromatography analysis of hypoxanthine incorporation into nucleotides in *E. tenella* oocyst (A) and chicken liver (B) extracts.

Table 2. Purine salvage enzymes in crude extracts of chicken liver and *E. tenella* unsporulated oocysts

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Specific activity, (nmol/min)/mg of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. tenella</em></td>
</tr>
<tr>
<td>HPRTase</td>
<td>0.48 ± 0.11*</td>
</tr>
<tr>
<td>GPRTase</td>
<td>0.77 ± 0.04*</td>
</tr>
<tr>
<td>XPRPase</td>
<td>&lt;0.1†</td>
</tr>
<tr>
<td>APRTase</td>
<td>0.66 ± 0.19*</td>
</tr>
<tr>
<td>Adenosine kinase</td>
<td>4.18 ± 1.40*</td>
</tr>
<tr>
<td>Xanthosine kinase</td>
<td>&lt;0.1†</td>
</tr>
<tr>
<td>Guanosine kinase</td>
<td>&lt;0.1†</td>
</tr>
<tr>
<td>Inosine kinase</td>
<td>&lt;0.1†</td>
</tr>
</tbody>
</table>

* Three independent trials.
† Six independent trials.
‡ Two independent trials.
affinity column XPRTase; n, of 22,400-23,200 purified C.4-sis (Fig. 5) allowed in have been the subunit represents the subunit of E. tenella HXGPRTase, the enzyme must have been purified to near homogeneity in one step by GMP-agarose affinity column chromatography. Inclusion of protein standards in the NaDodSO_4/polyacrylamide gel electrophoresis (Fig. 5) allowed an estimation of the molecular weight of the purified protein (30); results from two such runs gave a value of 22,400-23,200 daltons.

The purified enzyme can be stored in TMK buffer/10 mM PRPP/1 mM dithiothreitol at −196°C for weeks without appreciable loss of activity.

The chicken liver enzyme was partially purified by using a procedure for human erythrocyte HGPRTease (31). HPRTease and GPRTase activities copurified to a similar extent, suggesting that the chicken liver has a single enzyme HGPRTease.

Kinetic Studies of E. tenella HXGPRTase. For a saturating concentration of Mg^2+PRPP, initial rates were calculated from measurements made on reactions terminated 1, 2, and 4 min after initiation (Table 3). Data obtained within this time frame gave linear rates with a SEM of ±15%. Lineweaver-Burke plots of the data were then constructed by weighted least-squares analysis to produce various kinetic constants; for hypoxanthine, the value of K_i is lower and that of V_{max} is higher for the E. tenella enzyme than for chicken liver HGPRTease.

Allopurinol was tested on the two enzyme preparations and shown to be a relatively potent inhibitor of E. tenella HXGPRTase. Because of the structural similarity between allopurinol and the purine substrate, it was apparent that allopurinol is a competitive inhibitor and that initial velocity data at saturating concentrations of Mg^2+PRPP are adequately described by Eq. 1:

\[ v = A \times V_{max} / \left[ A + K_i \right] \times \left[ 1 + \frac{I}{K} \right] \]  

where v is the initial velocity, A is the concentration of substrate, I is the concentration of inhibitor. Initial velocity data obtained at various substrate and inhibitor concentrations were fit to this equation (32) by using a Gauss-Newton method of nonlinear regression in a commercially available statistical analysis package. The data gave allopurinol a K_i of 2.2 ± 0.9 \times 10^{-5} M versus hypoxanthine, which is ≈85 times higher than the K_i of hypoxanthine. Similar kinetic studies using xanthine or guanine as the substrate have been also carried out. The results (Table 3) suggest competition between allopurinol and the two other purines with similar K_i values, which could be also taken as an indication that there is but a single enzyme HXGPRTase in E. tenella. Allopurinol, however, has only a weak inhibitory effect on chicken liver HGPRTease. The compound has an apparent K_i of 9.3 ± 2.7 \times 10^{-5} M (Table 3), which is more than 40 times that for the E. tenella enzyme. Conversely, 6-mercaptopurine and 6-thioguanine were more potent inhibitors of chicken liver HGPRTease than of the E. tenella enzyme, the 50%
Table 3. Kinetic constants of E. tenella HXGPRTase and chicken liver HGPRTase

<table>
<thead>
<tr>
<th>Purine substrate</th>
<th>Crude extract</th>
<th>( V_{\text{max}} ) (pmol/sec)/mg of (allopurinol), protein</th>
<th>( K_s ) M</th>
<th>( K_i ) M \times 10^{-5}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoxanthine</td>
<td>E. tenella</td>
<td>1.4 ± 0.2 \times 10^{-6}</td>
<td>10.1 ± 0.4</td>
<td>93 ± 27</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>Chicken liver</td>
<td>2.5 ± 1.1 \times 10^{-7}</td>
<td>95.0 ± 2.8</td>
<td>2.2 ± 0.9</td>
</tr>
<tr>
<td>Guanine</td>
<td>E. tenella</td>
<td>3.7 ± 1.0 \times 10^{-7}</td>
<td>43.1 ± 2.7</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>Xanthine</td>
<td>E. tenella</td>
<td>1.5 ± 0.4 \times 10^{-6}</td>
<td>96.4 ± 10.8</td>
<td>1.2 ± 0.3</td>
</tr>
</tbody>
</table>

Results are mean ± SEM in the presence of 1.0 mM PRPP/7.0 mM MgCl₂. The \( K_s \) values for Mg²⁺ PRPP for the two enzymes are estimated to be 0.03–0.10 mM (unpublished observation).

inhibition concentrations being <1 \times 10^{-5} and 1.6 \times 10^{-5} M, respectively, against chicken liver versus 4.6 \times 10^{-5} M and 3.7 \times 10^{-5} M versus E. tenella HGPRTase activities. There is thus a difference in the inhibitor specificity between the host and the parasite enzymes.

DISCUSSION

This study has shown lack of change of labeled glycine and formate after prolonged incubation with extracts of E. tenella oocysts under conditions allowing expression of the activities of \( de novo \) purine synthesis in chicken liver extracts. Because glycine should be incorporated into purine nucleotides at the early stage of glycaminde ribonucleotide formation, whereas formate ought to be incorporated mostly into AICAR to make formyl AICAR toward the end of \( de novo \) purine synthesis, the lack of incorporation of both glycine and formate into purine nucleotides suggests absence of the entire \( de novo \) pathway in the extract of E. tenella oocysts. This finding provides a strong, although not conclusive, indication that E. tenella is incapable of \( de novo \) purine synthesis.

The presence of many purine salvage enzymes in E. tenella extracts at levels several times those in chicken liver extracts tends to support the argument that, because the parasite cannot make purines by itself, it has to have the ability to take up exogenous purines. HXGPRTase appears to be a crucial purine-salvaging enzyme for E. tenella, not only because of the abundant presence of hypoxanthine in its natural environment (19), but also because salvage of hypoxanthine can provide both AMP and CMP for the coccidia (11). The only other two purine-salvaging enzymes known to exist in E. tenella, APRTase and adenine kinase, can provide only AMP for the parasite. Due to the absence of AMP deaminase, there is no apparent way of converting AMP to GMP in E. tenella. The salvage of adenine and adenine alone thus may not fulfill the growth requirement by E. tenella; it may not even be essential.

No HGPRTase has ever been identified as a single enzyme in any other living organism. Cryptidia fasciculata, also sensitive to allopurinol in vitro, has HPRTase, GPRTase, and APRTase in three separate enzymes (33). L. donovani promastigotes contain three individual enzymes—HPRTase, XPRTase, and APRTase (19). E. tenella HGPRTase and L. donovani HGPRTase share some common properties in having relatively high \( v \) values (=7.0) (unpublished observation). The purification of E. tenella HXGPRTase to near homogeneity also shows that (i) the enzyme constitutes 0.14% of the total soluble protein in the cysts of E. tenella unsporulated oocysts; (ii) the enzyme subunit is smaller than that of human erythrocyte HPRTase, which has a molecular weight of 26,000 (31); (iii) the purified enzyme has an HPRTase specific activity that is one-fourth of that of purified human enzyme (31); and (iv) the enzyme has strong specific binding to GMP that cannot be dissociated by 0.1 M MgCl₂/0.5 M KCl at neutral pH.

The preferential inhibition of E. tenella HXGPRTase by allopurinol is interesting because the drug is known to inhibit the growth of E. tenella in cultured embryonic chicken kidney epithelial cells at 100 ppm (34). This in vitro anticoccidial activity of allopurinol may be due to its competitive inhibition of HXGPRTase, or by incorporation into the RNA of E. tenella. For the latter, one would have to assume that E. tenella adenylsucinate synthetase and lyase can also recognize allopurinol ribonucleotide and succinonaplopurinol ribonucleotide as their substrates, as is the case in L. donovani (33).

We wish to thank Dr. Herb Bull and Ms. Nancy Thornberry for their helpful discussions and nonlinear regression analysis of our enzyme kinetic data.