# ABSORPTION KINETICS OF SOME PURINES, PYRIMIDINES, AND NUCLEOSIDES IN *TAENIA CRASSICEPS* LARVAE

*by Gary L. Uglem and Michael G. Levy*

## ABSTRACT

*In vitro* kinetic studies revealed that *Taenia crassiceps* larvae absorb purine bases (adenine and hypoxanthine) and nucleosides (adenosine and uridine) by mediated transport. Pyrimidines (thymine, uracil, and cytosine) are absorbed primarily by diffusion. The presence of hypoxanthine stimulated adenosine uptake, an effect which was enhanced by preloading the worms with hypoxanthine. Furthermore, of the compounds tested, hypoxanthine alone displayed sigmoid absorption kinetics, suggesting an allosteric mechanism. This mechanism was repressed by preloading the worms with hypoxanthine, however, as well as by decreasing the incubation time from 2 minutes to 30 seconds. Under the latter experimental conditions hypoxanthine uptake appeared to occur by diffusion alone. The results suggest that the allosteric effects of hypoxanthine in this organism may involve an effect of hypoxanthine on metabolism and/or the adsorption of hypoxanthine and adenosine on cytoplasmic binding sites.

## INTRODUCTION

Radiographic experiments have shown that certain parasitic helminths rapidly incorporate absorbed purines and pyrimidines into nucleic acids (Prescott and Voge, 1959; Dvorak and Jones, 1963). Kinetic studies of the membrane transport of these bases are scarce, however, and are limited to studies of adult worms.

In this paper we describe two systems in *Taenia crassiceps* larvae for transporting nitrogenous bases and nucleosides. The allosteric nature of hypoxanthine absorption and the stimulatory effect of hypoxanthine on nucleoside uptake are also reported.

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## MATERIALS AND METHODS

Krebs-Ringer saline solution containing 25 mM tris(hydroxymethyl)-aminomethane-maleate buffer at pH 7.4 (KRT of Read et al., 1963) was the solution used in all washings, preincubations, and incubations. The labeled compounds used were adenine-8-$^{14}$C, thymine-2-$^{14}$C, adenosine-$^{3}$H(G), uridine-$^{3}$H(G) (Amersham Searle), uracil-2-$^{14}$C, hypoxanthine-8-$^{14}$C, and cytosine-2-$^{14}$C (New England Nuclear). Unlabeled compounds were obtained from Sigma and Calbiochem. Incubation media consisted of 5 ml of KRT containing a single labeled substrate with or without the appropriate unlabeled inhibitor.

Methods for maintaining laboratory infections of *Taenia crassiceps* larvae have been described previously (Pappas et al., 1973). Larvae were flushed from the body cavity of an infected mouse and washed several times. The small solid larvae were collected and distributed into random groups (40 to 80 mg wet wt/group). Each group of larvae was preincubated for 15 minutes (37° C) before incubation with the labeled substrate. Unless indicated otherwise, all incubations were for 2 minutes. To stop the incubations, larvae were washed briefly three times, blotted dry, and placed in 3 ml of 70% ethanol. After 24 hours the larvae were removed, dried for 24 hours at 95° C, and weighed. Radioactivity in aliquots of the ethanol extracts was determined using a scintillation spectrometer (Beckman). Data were compared using the Student's "t" test. The kinetic parameters ($V_{max}$ and $K_t$) were calculated using the Lineweaver-Burk analysis.

## RESULTS

*Taenia crassiceps* larvae absorbed $^{14}$C-thymine and -uracil in direct relation to substrate concentration; the worms were somewhat more permeable to thymine than to uracil as indicated by the diffusion rates (figure 1). Furthermore, at a low fixed substrate concentration (0.05 mM) the influx rates of these pyrimidines were not significantly inhibited by the addition of unlabeled molecules of the same species (figure 1, inset). Unlabeled cytosine (10 mM) inhibited the influx of 0.05 mM $^{14}$C-cytosine, indicating the presence of a small mediated component (not shown). A kinetic analysis of cytosine transport would be difficult, however, because of the large rate of entry by diffusion. Thus, the main route of entry for pyrimidines is by diffusion.

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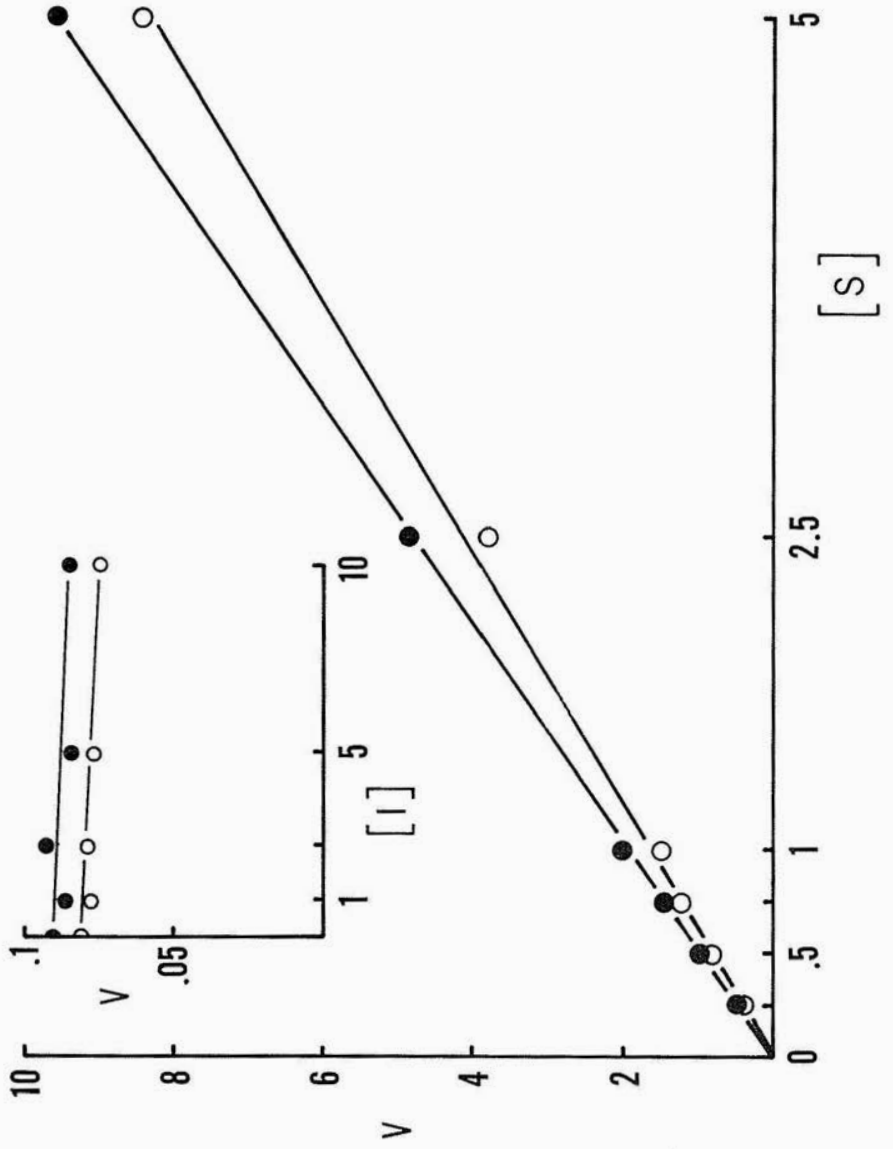
FIG. 1 (OPPOSITE). UPTAKE OF $^{14}$C-THYMINE (●) AND -URACIL (○) by *Taenia crassiceps* larvae. V = μmoles absorbed/g ethanol extracted dry wt/2 min. [S] = substrate concentration in mM. INSET: Uptake of 0.05 mM $^{14}$C-thymine (●) and -uracil (○) in the presence of unlabeled inhibitor of the same molecular species. [I]=inhibitor concentration in mM. Each point is the average of three samples.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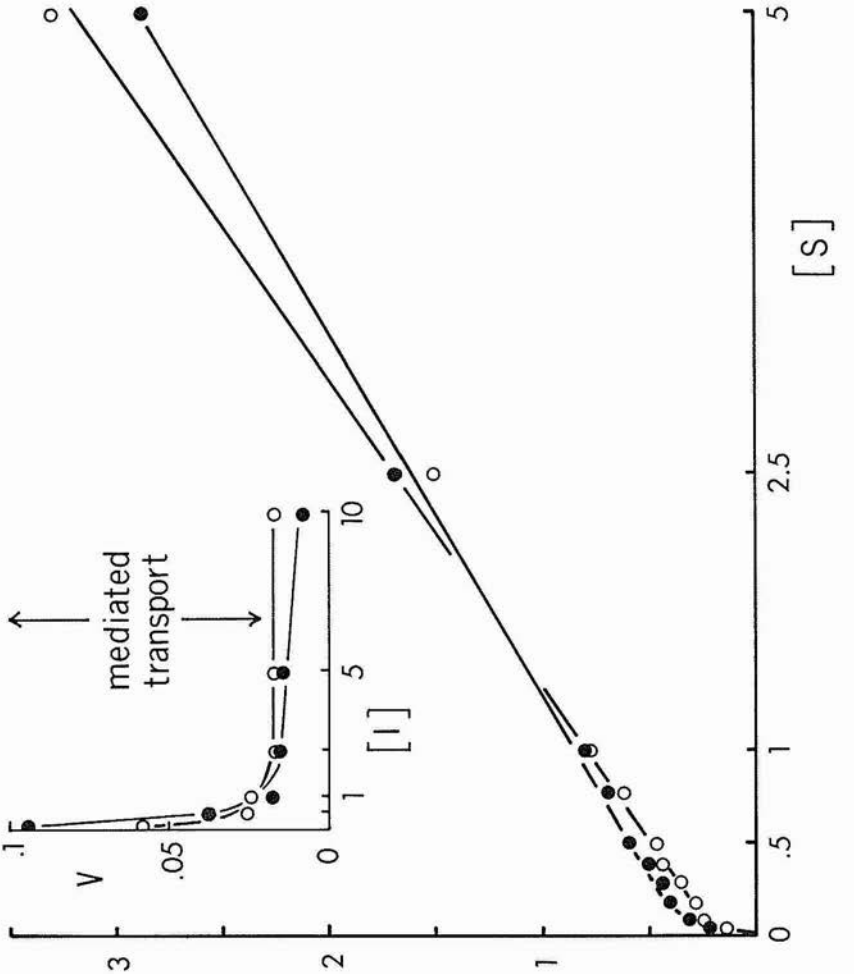


FIG. 2. UPTAKE OF $^3$H-URIDINE (●) AND -ADENOSINE (o) by *Taenia crassiceps* larvae. V = μmoles absorbed/g ethanol extracted dry wt/2 min. [S] = substrate concentration in mM. INSET: Uptake of 0.05 mM $^3$H-uridine (●) and -adenosine (o) in the presence of unlabeled inhibitor of the same molecular species. [I] = inhibitor concentration in mM. Each point is the average of three samples.

$^3$H-adenosine and -uridine entered larvae by a combination of mediated transport and diffusion (figure 2). Although the larvae appeared to be freely permeable to both compounds, mediated transport accounts for most of the uptake at 0.05 mM substrate concentration (figure 2, inset). Because of the small number of substrate concentrations tested below 0.2 mM, an accurate kinetic analysis could not be made. Nevertheless, the kinetic parameters for these nucleosides can be estimated by looking at the data in figure 2. The $V_{max}$ is between 0.17 and 0.3 $\mu$moles/g dry wt/2 min, while the $K_t$ is near 0.05 mM. Uridine transport appears to have a higher $V_{max}$, but a somewhat lower rate of entry by diffusion.

The effects of some purines and nucleotides on adenosine and uridine transport were observed (table 1). Hypoxanthine (6 mM) and adenine (10 mM) stimulated adenosine transport, while uridine transport was little affected by the compounds. If the worms were preincubated in 6 mM hypoxanthine for 15 minutes before incubation, hypoxanthine further stimulated adenosine transport. A maximum rate of stimulation was observed even when hypoxanthine was absent during incubation with labeled adenosine. AMP and ribose inhibited transport of these nucleosides.

TABLE 1

EFFECTS OF VARIOUS COMPOUNDS ON THE UPTAKE OF 0.05 mM $^3$H-ADENOSINE AND -URIDINE BY *TAENIA CRASSICEPS* LARVAE IN 2 MINUTE INCUBATIONS

The numbers in parentheses represent the percentage of inhibition (I) or stimulation (S). Uptake rates are expressed as $\mu$moles/g ethanol extracted dry wt/hr. Each value is the mean ± SE of three replicates. Values are uncorrected for diffusion.

| INHIBITOR (10 mM) | SUBSTRATE | | | |
|---|---|---|---|---|
| | Adenosine | | Uridine | |
| None | 1.62 ± 0.16 | — | 5.70 ± 0.01 | — |
| Adenosine | 0.35 ± 0.02 | (78% I) | 0.44 ± 0.05 | (92% I) |
| Uridine | 0.39 ± 0.06 | (76% I) | 0.35 ± 0.05 | (94% I) |
| Thymine | 1.11 ± 0.49 | (0) | 5.38 ± 0.63 | (0) |
| Uracil | 1.58 ± 0.10 | (0) | 4.88 ± 0.38 | (0) |
| Hypoxanthine (6 mM) | 3.14 ± 0.25 | (94% S) | 5.89 ± 0.11 | (0) |
| Adenine | 3.80 ± 0.72 | (134% S) | 6.39 ± 0.13 | (12% S) |
| AMP | 0.60 ± 0.32 | (63% I) | 1.97 ± 0.24 | (46% I) |
| Ribose | 0.97 ± 0.33 | (40% I) | 4.24 ± 0.26 | (26% I) |

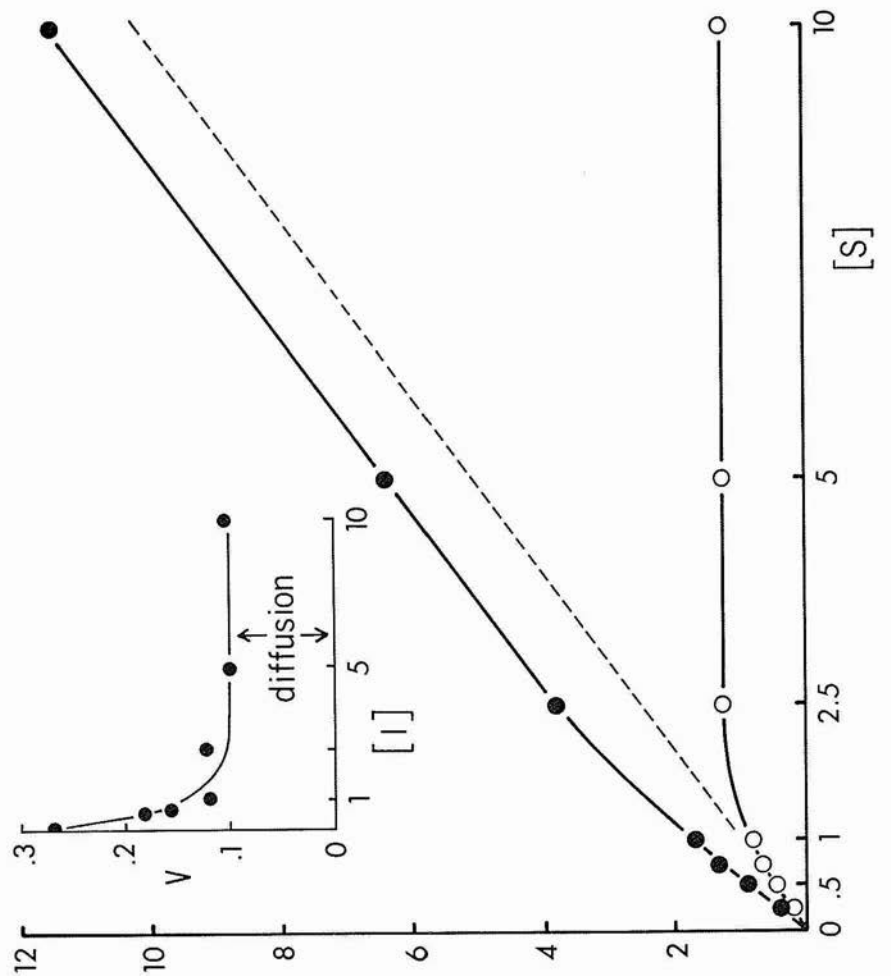


FIG. 3. UPTAKE OF $^{14}$C-ADENINE by *Taenia crassiceps* larvae. V = μmoles absorbed/g ethanol extracted dry wt/2 min. [S] = substrate concentration in mM. Dashed line represents the diffusion rate. Mediated transport (o) was calculated by subtracting diffusion from the observed rates (●). INSET: Uptake of 0.05 mM $^{14}$C-adenine in the presence of unlabeled adenine as inhibitor ([I] = mM). Each point is the average of three samples.

Two mediated systems appear to operate in the absorption of purines. The graph of $^{14}$C-adenine transport exhibited typical saturation kinetics (figure 3), with a rate of diffusion similar to that of the pyrimidines. Both the calculated $V_{max}$ (1.51 $\mu$moles/g dry wt/2 min) and $K_t$ (1.47 mM) were higher than those estimated for adenosine uptake. The rates of diffusion as determined from the V *vs.* [S] plot (figure 3) and the V *vs.* [I] plot (figure 3, inset) are in agreement. The fact that 10 mM unlabeled adenosine, uridine, or AMP did not affect the influx rate of 0.1 mM adenine indicates that adenine is transported at a separate site.

In contrast to adenine uptake, the rate of hypoxanthine uptake showed a sigmoid absorption pattern with respect to substrate concentration (figure 4), indicating the presence of an allosteric mechanism. Uptake appeared to be linear between 0.1 and 0.5 mM, was non-linear from 0.5 to 2.5 mM, and was again linear at concentrations greater than 2.5 mM. Unlabeled hypoxanthine (6 mM) inhibited the uptake of 0.05 mM $^{14}$C-hypoxanthine by 21% (figure 5, solid circles).

The above absorption pattern for hypoxanthine was changed by decreasing the incubation time. In 30 second incubations, the uptake rate was more linear with respect to increasing substrate concentrations (figure 6). Furthermore, the uptake of 0.05 mM $^{14}$C-hypoxanthine in 30 second incubations was not inhibited by unlabeled hypoxanthine, indicating that uptake is solely by diffusion (figure 6, inset). These patterns of absorption (i.e., linear V *vs.* [S] curve, and lack of inhibition in a V *vs.* [I] curve) could also be produced in 2 minute incubations by first preincubating the worms in 6 mM unlabeled hypoxanthine for 15 minutes before incubation with the substrate (figure 4, open circles; figure 5, open circles).

## DISCUSSION

Previous studies have suggested that purines and pyrimidines (MacInnis et al., 1965; MacInnis and Ridley, 1969; Pappas et al., 1973) and nucleosides (Page and MacInnis, 1975) move across the plasma membrane of *H. diminuta* by mediated transport. While several specific transport systems or loci have been identified for these compounds, the data are often difficult to interpret when the kinetic plots result in sigmoid-shaped curves. Furthermore, the presence of one compound might increase the rate of transport of another. To explain these apparent allosteric effects, the presence of two sites per locus has been postulated such that activation of the allosteric site increases the rate of translocation via the transport site. Although the molecular specificities of the allosteric systems in *H. diminuta* have been well defined, the significance of such systems is not clear.

We had hoped to determine the presence of mediated transport systems for nitrogenous bases in *T. crassiceps* larvae. The experiments demonstrated the

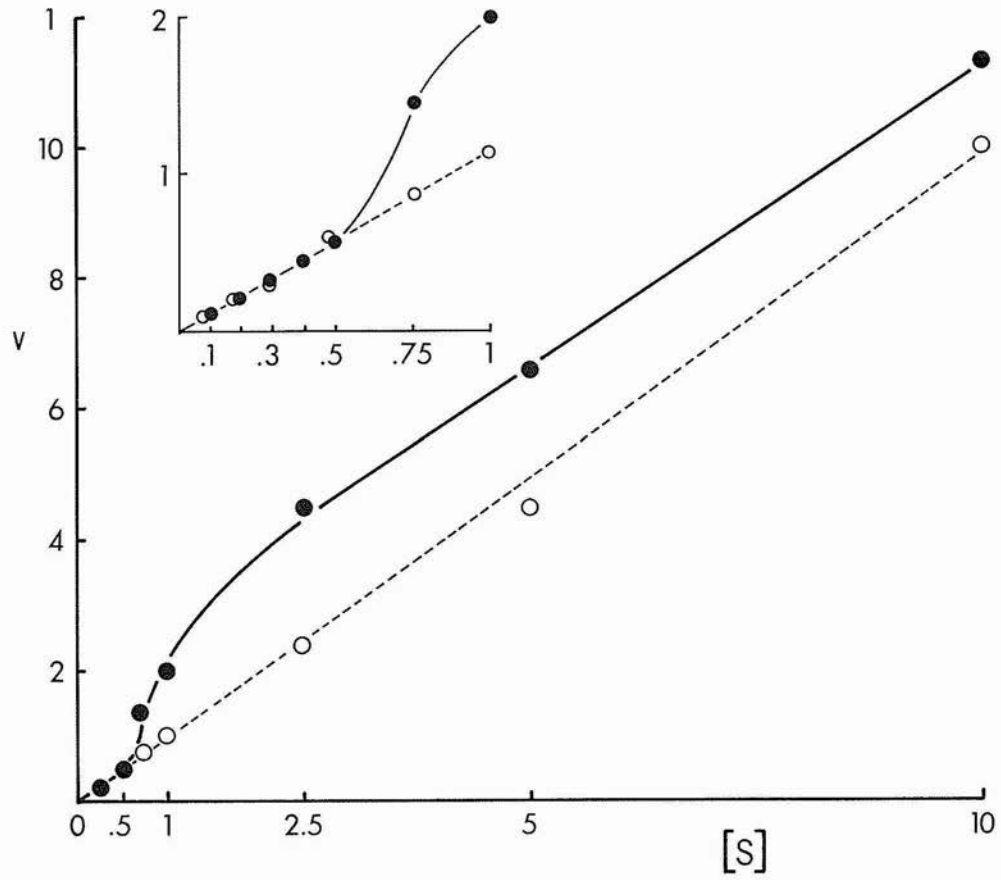


FIG. 4. UPTAKE OF $^{14}$C-HYPOXANTHINE (●) by *Taenia crassiceps* larvae. V = μmoles absorbed/g ethanol extracted dry wt/2 min. [S] = substrate concentration in mM. Some groups of worms (o) were preincubated with 6 mM unlabeled hypoxanthine for 15 minutes prior to incubation with the labeled substrate. Some of the points between 0.1 and 1.0 mM that are omitted for clarity are given in the inset. Each point is the average of three samples.

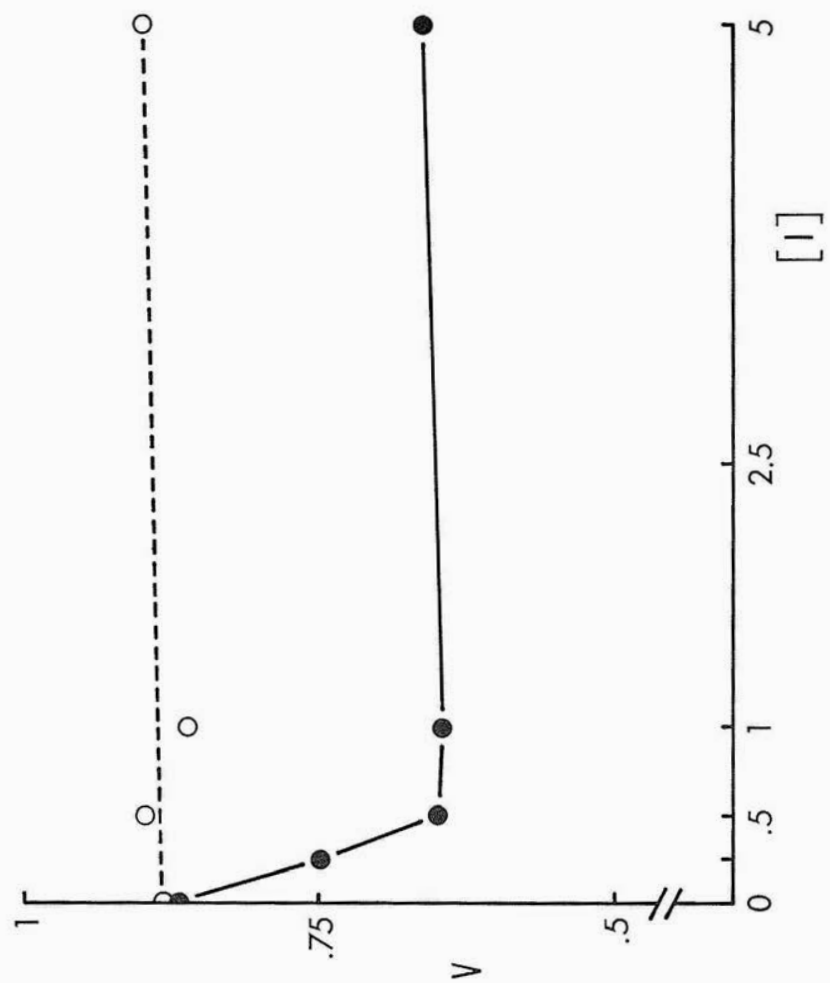


FIG. 5. UPTAKE OF 0.05 mM $^{14}$C-HYPOXANTHINE (●) by *Taenia crassiceps* larvae in the presence of unlabeled hypoxanthine as inhibitor ([I] = mM). V = μmoles absorbed/g ethanol extracted dry wt/2 min. Some worms (○) were preincubated with 6 mM unlabeled hypoxanthine for 15 minutes before incubation with the substrate. Each point is the average of three samples.

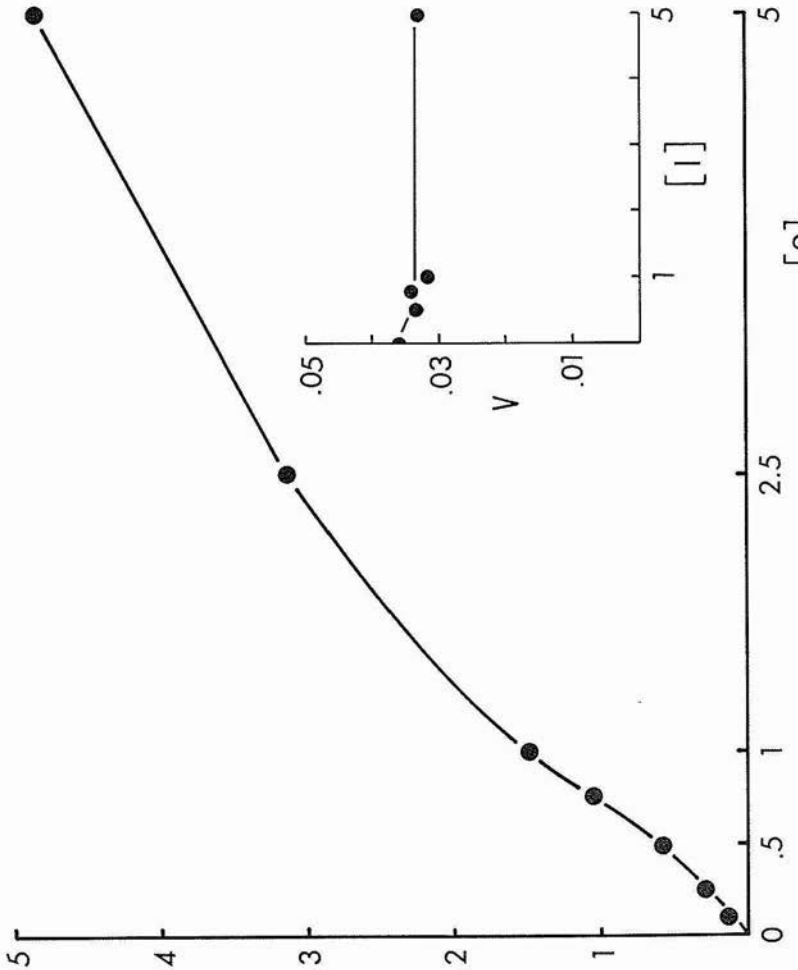


FIG. 6. UPTAKE OF $^{14}$C-HYPOXANTHINE by *Taenia crassiceps* larvae in 30 second incubations. V = μmoles absorbed/g ethanol extracted dry wt/30 sec. [S] = substrate concentration in mM. INSET: Uptake of 0.05 mM $^{14}$C-hypoxanthine in the presence of unlabeled hypoxanthine as inhibitor ([I] = mM). Each point is the average of three samples.

presence of such systems for purines and nucleosides. No allosteric system, however, such as exists in *H. diminuta*, was detected for pyrimidines in *T. crassiceps*. In fact, thymine and uracil appeared to be absorbed by diffusion alone. Until larvae of *H. diminuta* (or adults of *T. crassiceps*) are examined, we can only speculate whether the capacity for pyrimidine transport is characteristic of certain species or is a biochemical phenomenon found in larval but not adult cestodes.

While hypoxanthine stimulates adenosine uptake in both species of tapeworms, the mechanisms for absorbing hypoxanthine are different. In *H. diminuta* a plot of the velocity of uptake of hypoxanthine versus hypoxanthine concentration yields a typical saturation curve (MacInnis et al., 1965). Such a plot in *T. crassiceps* is distinctly sigmoidal. Furthermore, this effect in *T. crassiceps* is repressed by decreasing the incubation time to 30 seconds. Since binding of hypoxanthine to an allosteric site may not be significant in 30 seconds, some worms were preloaded first with hypoxanthine in an attempt to "prime" the system. Surprisingly, not only did this treatment fail to restore transport activity in 30 second incubations, but the allosteric pattern of uptake in 2 minutes was also repressed; the uptake of 0.05 mM labeled hypoxanthine was not inhibited by unlabeled hypoxanthine, and the V *versus* [S] plot was linear. On the other hand, hypoxanthine-loaded worms transported adenosine at higher rates even when hypoxanthine was not present in the incubation medium.

The interactions of purines and nucleosides in *T. crassiceps* are complex and difficult to explain by reference to membrane phenomena alone. If the allosteric effects of hypoxanthine prove to be due to the adsorption of hypoxanthine on sites within the cell (and if they in this way stimulate co-adsorption and/or metabolism of adenosine), then perhaps a more inclusive concept of transport and accumulation of nutrients in parasitic helminths is needed.

## ACKNOWLEDGMENT

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