tion, causes the formation of a distinct variety of this enzyme. The new allele, which was derived by mutation of the mutant allele am^3 , itself associated with apparent complete absence of glutamic dehydrogenase, is called am^{3a} .

2. The glutamic dehydrogenase associated with am^{3a} (the 3a enzyme) shows abnormally high Michaelis constants for all substrates, particularly for glutamate, triphosphopyridine nucleotide and NH_4^+ ion.

3. The ratio of maximum velocity in the reverse reaction (glutamate synthesis) to that in the forward reaction is about ten times as great for the 3a enzyme as for the wild-type enzyme. Extracts of am^{3a} strains have much higher maximum velocities in the reverse reaction than have wild-type extracts.

4. The 3a enzyme is less stable to heat than wild-type glutamic dehydrogenase.

5. In many preparations the 3a enzyme differs from the wild-type enzyme in not showing full activity until it has been given a mild heat treatment (e.g. 38° for a few minutes). This activation can also be brought about, to some extent, by incubation of the enzyme in the presence of α -oxoglutarate. Heat-activated 3a enzyme does not revert readily to the inactive form when returned to room temperature.

6. The main differences between the 3a and wild-type enzymes are just as marked in 40-fold-purified preparations as in crude extracts, though it was occasionally found that purified 3a preparations did not require activation. The two kinds of enzyme retained their respective properties when mixed together.

7. A heterocaryon containing both am^+ (i.e. wild type at the am locus) and am^{3a} nuclei produced glutamic dehydrogenase, which behaved as if it were a mixture of the 3a and wild-type enzymes.

8. Two mutant alleles at the am locus, each associated with a quite distinct abnormal type of glutamic dehydrogenase, are now known. Tests are described which distinguish the two mutant enzymes from each other, and from the wild type.

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Pyrimidine Metabolism in Parasitic Flatworms

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β -Alanine and β -aminoisobutyric acid have previously been reported as prominent free amino acids in several species of parasitic flatworms (Campbell, 1960a, b). These two β -amino acids were not detected in the free-living flatworms which were examined. Because of these findings it was thought that an investigation of the metabolic origin of these compounds might contribute to a better understanding of the biochemistry of parasitism.

When β -alanine and β -aminoisobutyric acid were first reported (Campbell, 1960a) from cestodes, it was suggested that they might arise from uracil and thymine respectively via the reductive pathway of pyrimidine degradation which has been established *in vivo* by Fink and co-workers (Fink, Henderson & Fink, 1952; Fink, Cline, Henderson & Fink, 1956; Fink, McGaughey, Cline & Fink, 1956; Fink, 1956) and *in vitro* by Grisolia and co-workers (Grisolia & Wallach, 1955; Wallach & Grisolia, 1957; Grisolia & Cardoso, 1957; Caravaca & Grisolia, 1958) and others (Canellakis, 1956;

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Fritzson, & Pihl, 1956; Campbell, 1958). Free pyrimidines, arising from the digestion of the nucleic acid (Wilson & Wilson, 1958) in the host's ingesta, are available to the intestinal parasites and possibly to all parasites in contact with host tissue, since pyrimidines may occur generally in tissues in the free form (Travaglini, Levenbook & Schultz, 1958).

In the present work it has been shown that uracil and thymine are taken up *in vitro* by the rat tapeworm, *Hymenolepis diminuta*, and metabolized to β -alanine and β -aminoisobutyric acid respectively via the reductive pathway of pyrimidine degradation. Two factors which affect the uptake and presumably the metabolism of these two compounds in this parasite are glucose and starvation of the host. Evidence is presented which indicates that α -[1- ^{14}C]alanine and a [^{14}C]organic acid, which is probably succinic acid, are end products of degradation of [2- ^{14}C]uracil by *H. diminuta*. The formation of these compounds is interpreted as being due to carbon dioxide fixation.

EXPERIMENTAL

The rat tapeworm was maintained in the laboratory in male rats of the Wistar strain (Read, 1951). Adult worms were flushed from the host's small intestine, after it had been removed from the body, with Krebs-Ringer solution (Umbreit, Burris & Stauffer, 1957), pH 7.4, with 0.05 M-2-amino-2-hydroxymethylpropane-1:3-diol (tris) acid maleate (Gomori, 1948). The wet weight of the individual worms averaged 316 mg. The average dry weight was approx. 18% of this. The percentage of nitrogen was 1.52 ± 0.02 , as determined on homogenates by a modification of the micro-Kjeldahl method (Lang, 1958).

Uptake of pyrimidines. The uptake of pyrimidines by individual parasites was studied by measuring the disappearance of these compounds from the medium during incubation. The worms were first incubated for 30 min.-1 hr. in Krebs-Ringer solution, pH 7.4, with 0.05 M-tris acid maleate buffer and then they were placed in the experimental flasks containing 10 ml. of the buffered solution plus the pyrimidine at 1 mM concentration. The flasks were stoppered with cotton wool plugs and the incubations were carried out at 37° with constant agitation. Samples of the medium were taken at appropriate times, diluted and the concentration of the pyrimidine was determined spectrophotometrically in the Beckman DU spectrophotometer. During the incubations the parasites elaborate a substance that absorbs ultraviolet light with an absorption spectrum which is essentially flat between 250 and 310 m μ . Partial compensation for this material was achieved by basing the calculations on the difference between $E_{\lambda \text{ max}}$ and either $E_{290 \text{ m}\mu}$ (for the pyrimidines) or $E_{304 \text{ m}\mu}$ (for orotic acid). Controls were also run in each experiment in which the pyrimidine was omitted from the incubation medium and the amount of absorption due to this material was determined and subtracted from the experimental values.

Cytosine, 5-hydroxymethylcytosine, 5-methylcytosine and orotic acid were obtained from the California Corporation for Biochemical Research. Uracil and thymine were

commercial preparations and were recrystallized twice from water.

Chromatography. β -Alanine and β -aminoisobutyric acid were extracted from the worms as described by Campbell (1960a, b). Two-dimensional chromatography of the free amino acid extracts was carried out on Whatman no. 52 paper by the method of Levy & Chung (1953), with the substitution of a butan-2-ol-formic acid-water (75:15:10, by vol.) system in the first dimension. The β -amino acids were estimated from these chromatograms by the method of Fowden (1951).

Uracil, dihydrouracil and carbamoyl- β -alanine were extracted from the parasites with 75% (v/v) ethanol. These compounds were separated by one- or two-dimensional ascending chromatography with Whatman no. 52 paper and the following solvent systems: butan-2-ol-formic acid-water, ethyl acetate-formic acid-water (60:5:35, by vol.; upper phase) and methanol-pyridine-water (80:4:20, by vol.). Uracil and dihydrouracil gave identical R_f values in all but the ethyl acetate system. These two compounds were separated in the other systems by eluting the uracil-dihydrouracil spot, hydrolysing it with 0.5 N-NaOH and re-chromatographing the hydrolysate to separate the original dihydrouracil from the uracil as carbamoyl- β -alanine. Dihydrouracil and carbamoyl- β -alanine were detected on the chromatograms by acidic *p*-dimethylaminobenzaldehyde by the method of Fink (1956).

Dihydrouracil and dihydrothymine were obtained from the California Corporation for Biochemical Research. Carbamoyl- β -alanine and carbamoyl- β -aminoisobutyric acid were synthesized from the corresponding dihydropyrimidine as described by Fink, McGaughey *et al.* (1956).

Ammonium salts of the organic acids were chromatographed on Whatman no. 1 paper. The solvent systems used were butan-2-ol-formic acid-water, methanol-pyridine-water, ethyl acetate-formic acid-water, propanol-conc. NH_3 sol. (70:30, v/v) and ethanol-conc. NH_3 soln.-water (80:5:15, by vol.). The indicator consisted of a mixture of methyl red and bromothymol blue in 7% formaldehyde (Block, Durrum & Zweig, 1958).

Isotopic techniques. [2- ^{14}C]Uracil was obtained from Schwarz Laboratories Inc., New York, U.S.A. Unlabelled uracil was added to this as a carrier to give the final concentrations used. For studies on the metabolism of uracil, the parasites were incubated in the [2- ^{14}C]uracil plus carrier as described above. The radioactive compounds formed from [2- ^{14}C]uracil were extracted and separated by chromatography as described above. They were detected on the chromatograms by No-Screen X-ray film (Eastman Kodak Co.) or by the use of a gas-flow chromatogram scanner. The amount of radioactivity in each compound was determined by eluting it from a chromatogram, plating the eluate on an aluminium planchet as a thin sample and counting the sample in a gas-flow counter. The $^{14}\text{CO}_2$ formed in the reactions was trapped in 10% (w/v) NaOH. It was then precipitated as BaCO_3 in the presence of NH_4Cl . An ethanolic slurry of the BaCO_3 was then plated as an infinitely thick sample and counted with a Geiger-Müller tube.

RESULTS

Uptake of pyrimidines by *Hymenolepis diminuta* *in vitro*. Of the six most commonly occurring pyrimidines, only uracil, thymine and cytosine were

taken up in significant quantities by the parasites. Uptake of orotic acid, 5-methyl- or 5-hydroxymethyl-cytosine was not detected (Fig. 1). The physiological state of the host influenced the rate at which uracil and thymine were taken up. When the hosts were starved 24 hr. before collection of the parasites, the uptake of uracil *in vitro* was approximately one-half of that of the parasites taken from hosts fed without restriction (Fig. 2). Since cestodes have a specific carbohydrate requirement and are sensitive to the host's dietary intake (Read, 1959), an attempt was made to reverse the effect of host starvation by the addition of glucose to the incubation medium. This resulted in an approximately twofold increase in uracil uptake by parasites taken from either starved or unstarved hosts (Fig. 2). Identical results were obtained with thymine. The presence of glucose in the incubation medium did not increase the uptake of cytosine whereas host starvation did. This is the reverse of the effects obtained with uracil and thymine and would suggest that a different mechanism may be involved in the metabolism of this pyrimidine.

Formation of β -alanine and β -aminoisobutyric acid from uracil and thymine respectively by Hymenolepis diminuta. Parasites freshly removed from the rat host contain about $0.6 \mu\text{mole}$ each of β -alanine and β -aminoisobutyric acid/g. of tissue. These con-

centrations remain relatively constant during incubation for 3–4 hr. in Krebs–Ringer solution, indicating that there is neither a very rapid turnover of these β -amino acids nor a high rate of their formation from endogenous sources. However, the concentrations of β -amino acids could be increased approximately five- to twenty-fold by incubating the parasites for 3 hr. in solutions of uracil, thymine, or the intermediate dihydropyrimidines or carbamoyl- β -amino acids (Table 1). The carbamoyl- β -amino acids were only approximately 50% as effective as precursors of β -alanine and β -aminoisobutyric acid when compared with uracil and dihydrouracil or thymine and dihydrothymine. This effect has previously been noted with rat-liver slices and has been explained on the basis of the lower permeability of the carbamoyl compounds into the intact cells (Fink, McGaughey *et al.* 1956).

Identification of the intermediates of degradation of $[2-^{14}\text{C}]$ uracil. The marked increase in the concentration of β -amino acids in *H. diminuta* after incubation in uracil and thymine, or of the intermediates of their degradation, strongly suggested that the reductive pathway of pyrimidine degradation was present. Evidence that dihydropyrimidines and carbamoyl- β -amino acids are the normal inter-

Fig. 1. Uptake of pyrimidines by *H. diminuta*. Each flask contained an individual parasite and $10 \mu\text{moles}$ of the pyrimidine in Krebs–Ringer solution, pH 7.4, with 0.05 M -tris acid maleate buffer. Incubations were at 37° with constant agitation. The concentrations of the pyrimidines during the incubation were determined spectrophotometrically as described in the text. ●, Uracil; ○, thymine; ▲, cytosine; △, 5-methylcytosine; ■, 5-hydroxymethylcytosine; □, orotic acid.

Fig. 2. Effect of host starvation on the uptake of uracil by *H. diminuta* and the reversal of this effect by glucose. Each flask contained an individual parasite and $10 \mu\text{moles}$ of uracil in Krebs–Ringer solution, pH 7.4, with 0.05 M -tris acid maleate. Uracil was determined spectrophotometrically as described in the text. ▲, Parasites collected from rats fed without restriction (unstarved hosts); ●, parasites collected from rats which had been fasted 24 hr. before the time of collection (starved hosts); △, parasites from unstarved hosts with 1 mg. of glucose/ml. added to incubation solution; ○, parasites from starved hosts with 1 mg. of glucose/ml. added to incubation solution.

Table 1. *Formation of β -amino acids from pyrimidines and the intermediates of pyrimidine degradation by Hymenolepis diminuta*

Parasites were pooled to give 1 g. of tissue/flask. Each flask contained 150 μ moles of the substrate in Krebs-Ringer solution, pH 7.4, with tris acid maleate. Incubations were at 37° for 3 hr. The reaction was stopped with ethanol and the β -amino acids were extracted as described in the text. Results are given $\pm$ S.E.M. with the number of determinations in parentheses.

Treatment	Substrate	β -Alanine (μ moles/g. of tissue)	β -Aminoisobutyric acid (μ moles/g. of tissue)
Freshly removed from host	—	0.60 $\pm$ 0.04 (8)	0.65 $\pm$ 0.08 (8)
Incubated for 3 hr. in Krebs-Ringer solution	—	0.85 $\pm$ 0.13 (8)	0.63 $\pm$ 0.08 (8)
Killed by heat (5 min. at 80°)	Each substrate tested*	0.95 $\pm$ 0.40 (6)	0.68 $\pm$ 0.25 (6)
Incubated for 3 hr.	Uracil	16.90 $\pm$ 0.29 (4)	1.50 $\pm$ 0.48 (4)
	Dihydrouracil	19.30 $\pm$ 0.93 (4)	1.10 $\pm$ 0.01 (4)
	Carbamoyl- β -alanine	6.80 $\pm$ 0.60 (4)	0.60 $\pm$ 0.01 (4)
	Thymine	0.90 $\pm$ 0.01 (4)	11.10 $\pm$ 0.50 (4)
	Dihydrothymine	1.50 $\pm$ 0.01 (4)	10.50 $\pm$ 0.63 (4)
	Carbamoyl- β -aminoisobutyric acid	0.80 $\pm$ 0.01 (4)	4.30 $\pm$ 0.35 (4)

* These controls consisted of heat-killed parasites plus each substrate tested. Since there was little variation, the data were pooled from all the experiments for statistical analyses.

Table 2. *R_F values on Whatman no. 52 paper of known dihydrouracil and carbamoyl- β -alanine and two radioactive compounds formed from [2-¹⁴C]uracil by Hymenolepis diminuta*

Solvent	R _F	
	Dihydro-uracil	Carbamoyl- β -alanine
Butan-2-ol-formic acid-water (75:15:10, by vol.)	0.63	0.76
Ethyl acetate-formic acid-water (60:5:35, by vol.; upper phase)	0.45	0.52
Methanol-pyridine-water (80:4:20, by vol.)	0.54	0.63

mediates between the pyrimidines and the β -amino acids involved the chromatographic isolation and identification of radioactive dihydrouracil and carbamoyl- β -alanine after incubation of the parasites in [2-¹⁴C]uracil. This was carried out in a typical experiment as follows. The parasites were pooled to give approximately 1 g. of tissue/flask. They were then incubated for 3 hr. at 37° in Krebs-Ringer solution, pH 7.4, containing 30 μ moles of [2-¹⁴C]uracil (specific activity 31828 counts/min./ μ mole). At the end of the incubation they were quickly removed from the flask, washed and homogenized in 3 ml. of 75% (v/v) ethanol. The precipitate was removed by centrifuging and 200 μ l. of this supernatant was spotted on to Whatman no. 52 paper for chromatography. Since the concentrations of dihydrouracil and carbamoyl- β -alanine were low in these extracts, only faint colours were given with acidic *p*-dimethylaminobenzaldehyde. To identify these compounds, 200 μ l. of the extract to which had been added 1–2 μ moles each of known dihydro-

uracil and carbamoyl- β -alanine was chromatographed alongside the untreated extracts. The chromatogram was then exposed to X-ray film for several days. After the radioautograph had been developed, the chromatogram was sprayed with *p*-dimethylaminobenzaldehyde to detect the carbamoyl- β -alanine. It was then treated with 0.5N NaOH and resprayed with *p*-dimethylaminobenzaldehyde to locate the dihydrouracil. This procedure was repeated with three different solvent systems and the R_F values of known dihydrouracil and carbamoyl- β -alanine and the radioactive compounds in the extracts, with identical properties with respect to their reaction with *p*-dimethylaminobenzaldehyde, are given in Table 2. The colour reaction of these compounds on the chromatograms and the radioactivity were found to coincide perfectly; tracings of typical radioautographs of the extracts and of the incubation medium are presented in Fig. 3.

Identification of the end products of degradation of [2-¹⁴C]uracil. The formation of ¹⁴CO₂ from [2-¹⁴C]uracil has previously been used as a criterion for the reductive degradation of uracil (Canellakis, 1956). As shown in Fig. 4, this also takes place in *H. diminuta*.

In addition to carbon dioxide, dihydrouracil and carbamoyl- β -alanine, there are two other major radioactive compounds formed from [2-¹⁴C]uracil, which, as described below, appear to be α -alanine and succinic acid. As shown in Table 3 and Fig. 5, these two compounds together account for approximately 60% of the recovered radioactivity in the extracts after incubation for 3 hr. The compound designated as α -alanine was separated from the β -alanine by streaking 300–500 μ l. portions of the

extracts on Whatman no. 52 paper and developing the paper with butan-2-ol-formic acid-water. Radioautographs were then made and the radioactive area was eluted. The eluate was concentrated and portions were chromatographed with and without known α -alanine being added to them. Known

α -alanine was also run alongside these extracts. Radioautographs were then made of these chromatograms and the radioactivity and ninhydrin reaction were found to coincide. The R_f values obtained for known alanine and the alanine isolated from the worm extracts are given in Table 4.

Table 3. *Distribution of the radioactivity from [2- 14 C]uracil between the compounds, isolated from extracts of Hymenolepis diminuta*

After incubation for 3 hr. in [2- 14 C]uracil the parasites were extracted and the radioactive compounds formed were isolated by chromatography as described in the text. The percentage of the radioactivity present in each compound was determined by eluting the compound from a one-dimensional chromatogram and counting a sample of the eluate with a gas-flow counter.

Compound	Radioactivity (%)		
	Butan-2-ol-formic acid-water	Ethyl acetate-formic acid-water	Methanol-pyridine-water
Alanine	15.1	15.9	15.2
'Succinic acid'	43.9	43.4	44.1
Carbamoyl- β -alanine	4.1	3.9	4.1
Dihydrouracil	5.1	4.2	4.5
Uracil	31.3	32.0	31.3
Unaccounted for	0.5	0.6	0.8

Fig. 3. Tracings of radioautographs made from chromatograms of the 75% ethanol extracts of parasites incubated in [2- 14 C]uracil and of the incubation medium. Above each chromatogram is a tracing of the record obtained from a gas-flow chromatogram scanner which indicates the approximate percentage of the total recovered radioactivity in each compound. The extraction and chromatography is as described in the text. (a), (b) and (d) represent extracts chromatographed in three solvent systems: ethyl acetate-formic acid-water (EAFW), butan-2-ol-formic acid-water (BFW) and methanol-pyridine-water (MPW). (c) represents a sample of the incubation medium and (e) an extract of parasites which had been killed by heating (5 min. at 80°). ALA, α -Alanine; URA, uracil; DHU, dihydrouracil; c- β -ALA, carbamoyl- β -alanine; OA, organic acid; X, unidentified compounds which were not consistently detected.

Fig. 4. Formation of $^{14}\text{CO}_2$ from $[2\text{-}^{14}\text{C}]\text{uracil}$ by *H. diminuta*. The reaction was carried out in Warburg vessels containing 10% NaOH in the centre well. At time 0, 0.75 ml. of 0.02 M $[2\text{-}^{14}\text{C}]\text{uracil}$ was added to the incubation medium (total volume 3.25 ml.) and the incubation was carried out at 37° . The reaction was stopped at the indicated time intervals with 10% (w/v) trichloroacetic acid. The trapped CO_2 was precipitated as BaCO_3 and counted with a Geiger-Müller tube. ●, Experimental; ▲, killed by heat (5 min. at 80°).

Fig. 5. Percentage of the radioactivity in extracts of *H. diminuta* present in uracil, dihydrouracil, carbamoyl- β -alanine, alanine and 'succinic acid' during incubation for 3 hr. in $[2\text{-}^{14}\text{C}]\text{uracil}$. The conditions of the incubation and extraction of the compounds are as described in the text. The percentage of the radioactivity in each compound was determined by eluting the compound from a one-dimensional chromatogram (developed with ethyl acetate-formic acid-water) and counting a sample of the eluate with a gas-flow counter. ▲, Uracil; □, 'succinic acid'; ●, α -alanine; △, dihydrouracil; ○, carbamoyl- β -alanine.

The radioactivity of the alanine was localized in the carboxyl group in the following manner. An eluate containing 2200 counts/min. of the alanine in 2 ml. was caused to react with 1 ml. of Fowden's (1951) ninhydrin reagent at pH 5.0, in a closed vessel at 100° for 30 min. The CO_2 formed was trapped in 10% NaOH and 2036 counts/min. were recovered as $\text{Ba}^{14}\text{CO}_3$.

Preliminary experiments indicated that the compound which accounts for over 40% of the recovered radioactivity in the extracts after incubation for 3 hr. in $[2\text{-}^{14}\text{C}]\text{uracil}$ was an organic acid (designated as OA in Fig. 3). This compound is the first compound in which activity could be detected. It was easily detectable by radioautography after incubation for 5 min. This compound was chromatographically isolated as described above for the alanine. Chromatographic comparisons with several known acids suggested that it might be succinic acid. Portions of the eluate were neutralized with NH_3 solution and were chromatographed with and without added succinic acid neutralized in the same manner. When radioautographs of these were prepared, the radioactivity and indicator reaction were found to coincide exactly. The R_f values of the radioactive organic acid isolated from the parasite extracts and known succinic acid are given in Table 5.

Table 4. R_f values on Whatman no. 52 paper of known α -alanine and radioactive α -alanine isolated from extracts of *Hymenolepis diminuta* after incubation in $[2\text{-}^{14}\text{C}]\text{uracil}$

Solvent	R_f
<i>m</i> -Cresol-phenol-borate buffer, pH 9.3 (60:30:15, by vol.)	0.36
Butan-2-ol-formic acid-water (75:15:10, by vol.)	0.45
Ethyl acetate-formic acid-water (60:5:35, by vol.; upper phase)	0.02
Methanol-pyridine-water (80:4:20, by vol.)	0.61
Propanol-conc. NH_3 soln. (70:30, v/v)	0.51

Table 5. R_f values on Whatman no. 1 paper of known succinic acid and the radioactive organic acid isolated from *Hymenolepis diminuta* after incubation in $[2\text{-}^{14}\text{C}]\text{uracil}$

Solvent	R_f
Propanol-conc. NH_3 soln. (70:30, v/v)	0.14
Ethanol-conc. NH_3 soln.-water (80:5:15, by vol.)	0.18
Butan-2-ol-formic acid-water (75:15:10, by vol.)	0.75
Ethyl acetate-formic acid-water (60:5:35, by vol.; upper phase)	0.81
Methanol-pyridine-water (80:5:20, by vol.)	0.25

Traces of other radioactive compounds were occasionally detected on radioautographs of the parasite extracts (Fig. 3, X) but were not identified. [^{14}C]Urea is one of the major products from [$2\text{-}^{14}\text{C}$]thymine in the rat (Fink, McGaughey *et al.* 1956). To determine if any [^{14}C]urea was formed by these parasites from [$2\text{-}^{14}\text{C}$]uracil, all the radioactive spots on the chromatograms were eluted and treated with urease. No $^{14}\text{CO}_2$ production was detected with any of the spots whereas [^{14}C]urea added to the worm extracts and carried through the same procedure of chromatography, elution and urease treatment resulted in the formation of $^{14}\text{CO}_2$.

DISCUSSION

The evidence presented indicates that reductive degradation of uracil and thymine via either dihydrouracil and carbamoyl- β -alanine or dihydrothymine and carbamoyl- β -aminoisobutyric acid to form β -alanine or β -aminoisobutyric acid respectively is an important pathway in the rat parasite *H. diminuta*. That this pathway may be present in several other parasitic flatworms is indicated by the rather wide occurrence of β -alanine and β -aminoisobutyric acid in these forms (Campbell, 1960*a*, *b*).

Previous work with the reductive pathway of thymine degradation *in vivo* by Fink and co-workers (Fink *et al.* 1952; Fink, McGaughey *et al.* 1956; Fink, Cline *et al.* 1956) has shown that, in rat-liver slices, various radioactive compounds are formed, in addition to dihydrothymine and carbamoyl- β -aminoisobutyric acid, depending upon the position of the radioactive carbon in the thymine molecule. The radioactive compounds identified from methyl-labelled thymine were 5-hydroxymethyluracil, alanine and glucose. With [$2\text{-}^{14}\text{C}$]thymine these investigators have reported radioactive 5-hydroxymethyluracil, urea, uracil-5-carboxylic acid and carbon dioxide. In *H. diminuta*, $^{14}\text{CO}_2$, $\alpha\text{-}[1\text{-}^{14}\text{C}]$ -alanine and a [^{14}C]organic acid, which is probably succinic acid and will be referred to as such subsequently, are the major end products identified from metabolism of [$2\text{-}^{14}\text{C}$]uracil. The most tenable explanation for the formation of the last-named two compounds involves a mechanism of carbon dioxide fixation. Carbon dioxide fixation by animal parasites has previously been reported by Fairbairn (1954) in the roundworm of chickens, *Heterakis gallinae*, and more recently by Saz & Vidrine (1959) in muscle of the roundworm of swine, *Ascaris lumbricoides*. *Heterakis* was found to fix carbon dioxide into propionic acid and a non-volatile acid which is probably succinic acid. In *Ascaris* muscle, the mechanism involves an initial fixation into pyruvic acid followed by a reduction to form succinic acid. It was also found that in *Ascaris* muscle succinic acid may be decarboxylated to form pro-

pionic acid. Hammen & Wilbur (1959) have shown that in the oyster there is an initial fixation of carbon dioxide into propionic acid to form succinic acid. The mechanism of the formation of the [^{14}C]-succinic acid and $\alpha\text{-}[1\text{-}^{14}\text{C}]$ alanine in *H. diminuta* from [$2\text{-}^{14}\text{C}$]uracil is at present not known. The fixation of $^{14}\text{CO}_2$ from the uracil into the carboxyl group of the succinic acid followed by oxidation and decarboxylation to form pyruvic acid is one pathway which could account for the labelling of the carboxyl group of alanine, since the pyruvic acid $\rightleftharpoons$ alanine transaminase system is present in this parasite (Aldrich, Chandler & Daugherty, 1954).

The increased uptake of uracil or thymine in the presence of glucose might be due to an increased metabolism of these compounds. Glucose did not increase the uptake of cytosine, which is apparently not degraded by animal tissues (Fink, McGaughey *et al.* 1956). Read (1956) has shown that glucose increases both the oxygen consumption and lactic acid production by these parasites. Host starvation results in a greatly diminished polysaccharide content and a decreased rate of acid production. The amount of acid produced is as follows: parasites from starved hosts plus glucose > from unstarved hosts plus glucose > from unstarved hosts > from starved hosts. Host starvation was also found to increase the amount of glycogen synthesis by the parasites in the presence of glucose (Read, 1956; Read & Rothman, 1957). Although parasites from starved hosts produce more lactic acid in the presence of glucose, they have a lower rate of glucose uptake (Phifer, 1960). The uptake of uracil then corresponds to the rate of glucose uptake. The time lag before glucose has its effect (Fig. 2) indicates that the increased rate of uracil uptake may be dependent upon glucose metabolism. Preliminary experiments show that uracil is not transported against a concentration gradient by *H. diminuta*. Similar results have been obtained with rat and hamster intestinal preparations (Wilson & Wilson, 1958). This indicates that the effect of glucose and host starvation reflects an effect on the metabolism of this compound and not on some transport mechanism. This effect may be one of an increased or decreased supply of reducing compounds or adenosine triphosphate.

SUMMARY

1. The rat tapeworm, *Hymenolepis diminuta*, has been found to take up uracil, thymine and cytosine *in vitro*. The addition of glucose to the medium results in a marked increase in the uptake of uracil and thymine, but not of cytosine.

2. Uracil and thymine are degraded to β -alanine and β -aminoisobutyric acid respectively by this parasite. The degradation of [$2\text{-}^{14}\text{C}$]uracil results in

the formation of radioactive dihydrouracil, carbamoyl- β -alanine, carbon dioxide and, in addition, α -[1- ^{14}C]alanine, some unidentified compounds and an organic acid which is chromatographically identical with succinic acid.

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Immunological Properties of Exopenicillinase Synthesized by *Bacillus cereus* 569/H in the Presence of Amino Acid Analogues

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Previous experiments with *Staphylococcus aureus* 524 have shown that the replacement in the growth medium of the essential amino acid, arginine, by the structural analogue canavanine led to continued protein synthesis, but no production of active forms of phosphatase, β -galactosidase, hyaluronidase nor of a lytic enzyme which digested cell-wall preparations from *Micrococcus lysodeikticus*. To explain this finding it was suggested that protein synthesized in the presence of canavanine contained that amino acid in place of arginine and that this resulted in inactive forms of the enzymes (Richmond, 1959a). This possibility was supported by the detection of canavanine in the protein

fraction of organisms incubated under these conditions (Richmond, 1959b).

It would seem that the one feasible method of testing this hypothesis would be by detecting an immunological cross-reaction between the inactive enzyme protein and a specific antibody against the normal enzyme. Unfortunately specific antisera against the enzymes studied in *S. aureus* were not available nor did it seem practicable to purify these enzymes to the degree necessary for the preparation of such sera. Accordingly, the effect of canavanine was studied on penicillinase formation by *Bacillus cereus* 569 (inducible strain) and *B. cereus* 569/H (constitutive strain). This system was