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IN VITRO AND IN VIVO GLYCINE ABSORPTION BY TWO SPECIES OF *HYMENOLEPIS* (CESTOIDEA)

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IN VITRO AND IN VIVO GLYCINE ABSORPTION BY TWO SPECIES OF

HYMENOLEPIS (CESTOIDEA)

APPROVED BY

DISSERTATION COMMITTEE

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CHAPTER I

INTRODUCTION

Knowledge of the biochemical and physiological relationships between cestodes and their hosts has advanced rapidly during the past quarter-century. Timely reviews of research in this field are those of McCoy (1935), Wardle (1937), Smyth (1947), von Brand (1948, 1952, 1957), Bueding (1949, 1950), Bueding and Most (1953), Read (1960), and Read and Simmons (1963). Investigations on cestode metabolism of nitrogenous compounds have not been as extensive as have those of carbohydrate metabolism; nevertheless, some very significant and detailed information has been gained. Adult cestodes are noted for having a relatively low protein content, considerable nonprotein nitrogen, a high rate of protein synthesis, and high nitrogen turnover. Furthermore, the nitrogen content per weight of tissue decreases along the length of cestodes from the scolex toward the terminal proglottid (Fairbairn, et al., 1961).

Apparently, dry matter varies significantly between orders of tapeworms. Hopkins and Hutchison (1960) reported the dry matter of adult cyclophyllideans (an order found mainly in birds and mammals)

to be about 20 to 25% of the wet wt., while it is about 30% of the wet wt. in adult pseudophyllideans from warm-blooded hosts. The determinations reported by Hopkins and Hutchison have for the most part been confirmed in more recent investigations. Haley (1962) called attention to some factors causing variations in metabolic analyses; some of these reflect species, strain, and age differences. The number of cestodes infecting the host, coinfection with another species of parasite, diet, general physiological condition of the host, previous exposure to infection, and the methodology employed by the individual investigator all can alter physiological and biochemical analyses. Each of these factors is believed to affect not only the size, weight, morphology, and normal development of cestodes, but also the worm's metabolic capability.

In a series of experiments in which rats infected with Hymenolepis diminuta were fed a protein free diet, Chandler (1943) demonstrated that this tapeworm is independent of the host dietary protein, and one would presume that it synthesizes its own protein from host amino acids. Certainly a high rate of protein synthesis and nitrogen turnover in tapeworms is suggested by their rapid growth and development, and Chandler's observations raise questions regarding the source and means by which the nitrogen requirements of these parasites are satisfied.

Campbell's (1960) analysis of the nitrogen fraction of three species of anoplocephalids, and a study of the free amino acids of Dipylidium caninum by Hopper (1959) have shown tapeworm tissue to contain most, if not all, of the naturally occurring amino acids. Goodchild and Wells (1957) demonstrated a qualitative parallel between the protein amino acids found in the larvae and adults in H. diminuta

and those of the beetle and rat host tissues with which these stages are associated. They found also, that there is a quantitative decrease in the protein amino acids in adult worms kept in Thiry-Vella fistulas of the rat small intestine for three days. They interpreted this to mean that amino acid incorporation into protein is inhibited in the absence of bile salts.

Qualitative differences in the free amino acid complex (Aldrich, et al., 1954; Garson, 1954; Foster, 1955; Foster and Daugherty, 1957, 1959; Campbell, 1963) and the transaminase activities (Aldrich, et al., 1954; Foster and Daugherty, 1957, 1959) of H. diminuta and Raillietina cesticillus and the differences in the transaminase activities of H. diminuta, H. citelli, and H. nana (Wertheim, et al., 1960) lend presumptive evidence for the absorption of intact molecules of most amino acids providing the nitrogen requirement of cestodes.

Campbell demonstrated that both the sheep tapeworms, Moniezia expansa from the intestine, and Thysanosoma actinioides from the gall bladder and bile passages, contain qualitatively the same protein amino acids. He further showed that sheep bile is rich in both free and protein amino acids and that, with two exceptions, the bile contains qualitatively the same amino acids as do the cestodes. The exceptions were the aminobutyric acids which are present in all the cestodes and histidine which was present in T. actinioides. These amino acids were not found in the sheep bile. T. actinioides contained 10% more dry weight protein than did M. expansa. Campbell concluded that the bile could supply the amino acid requirement of T. actinioides.

In unpublished work I have found that all of the free amino acids of D. caninum are present in dog bile. More than 30 nitrogen containing

components of the free amino acid pool of this cestode were detected with refined techniques. The protein amino acids of tapeworms are somewhat fewer in number, and as Kent (1957) has shown, the amino acid residues of proteins prepared by identical methods are quite variable between species.

According to Read (1950a, 1955), Read and Simmons (1963), and Read, et al. (1963), the total contribution of nitrogenous materials from the mucosa and glandular organs associated with the small intestine of vertebrates, together with the products of synthesis and degradation by the lumen microorganisms, is quite sufficient to swamp and mask the dietary contribution. In consideration of the secretions, absorptions, resecretions, reabsorptions, and synthetic and degradation changes of substances reaching the lumen, he has termed the overall mechanism by which the total nondietary gut contents reaches the lumen of the small intestine the "exocrino-enteric circulation." Further, he terms the physicochemical relationships between a host and parasite, involving a region in space and time, the "host-parasite interface."

A great variety of biological compounds are presented to the host-parasite interface by means of the exocrino-enteric circulation, and even under deprivation of protein from the diet, amino acids are present in definite constant ratios in the lumen of the small intestine (Nasset and Ju, 1961). Some compounds occurring at this interface may arrive there in a host secretion, yet not be of host origin. They may be formed by the action of the host's microorganisms, absorbed by the host, and then secreted back into the gut via the bile. Deoxycholic acid, a compound in the gall bladder bile of several mammals, is reported to be formed in this fashion (Smyth and Haslewood, 1963).

It has been demonstrated that bile and bile salts have important effects on excystation, growth, development, and maintenance of cestodes in the host. Whole bile (in addition to water, mucin, inorganic ions, amino acids, and carbohydrates) contains mainly pigments, a variety of lipids, and bile acids. The bile acids are usually conjugated with either glycine (herbivores) or taurine (carnivores) or both. Gall bladder bile is more viscous, and may be up to nine times more concentrated in some compounds, than hepatic bile. The number, kind, and relative concentration of bile acids in bile is quite variable between species, and variations in an individual are related to the state of health.

Smyth and Haslewood (1963) demonstrated that deoxycholic acid, chenodeoxycholic acid, and their glycine and taurine conjugates lyse protoscolices of Echinococcus granulosus. They also demonstrated that these compounds were not present in sufficient concentration in dog bile to cause lysis of the protoscolices. However, these compounds were demonstrated to be present in sufficient concentration in the bile of several animals including man to cause lysis. Smyth and Haslewood also demonstrated that commercial bile salts contain lysis-producing contamination and point out the importance of using purified samples in experimental work. They suggest that "bile" may act as a powerful selective agent in determining host specificity in intestinal parasitism. Specific bile salts may act as biochemical agents by: 1) stimulating egg, cyst, or spore hatching; 2) lysing, and thus eliminating parasites from unsuitable hosts; and 3) stimulating parasite metabolism, which results in the establishment, growth, development, and reproduction in suitable hosts.

Rothman (1958) demonstrated that the sodium salts of cholic, taurocholic, and glycocholic acids inhibit the anaerobic fermentation of glucose at pH 7 in H. diminuta and Oochoristica symmetrica. He also demonstrated that sodium taurocholate inhibits glucose metabolism in H. diminuta from both rats and hamsters, but not in H. citelli from hamsters or Taenia taeniaformis from cats (Rothman, 1959). In T. crassiceps larvae the endogenous metabolism was stimulated, as measured by scolex evagination and activity. Phifer (1960) reported that a 15 minute pretreatment of H. diminuta with taurocholate produces a fourfold inhibition of the uptake of C^{14} labeled glucose.

Litchford (1963), after successfully infecting hamsters, young rats, and mice with H. microstoma, suggested that the size attained in the different hosts was possibly attributable to the chemistry of the host bile.

Goodchild (1958a, 1958b, 1960, 1961a, 1961b) showed that when rats are deprived of bile, there is a depression of 1) development of cysticercoids into adults, 2) growth and sexual maturation of transplanted adults, and 3) altered percentage composition of water, carbohydrate, glycogen, and protein, in H. diminuta. He concluded that deprivation of bile is more detrimental to the cestodes than starvation of the host. Vilar-Alvarez and Goodchild (1961) reported that H. diminuta failed to survive after transfer into small intestine resections deprived of bile. Later, Goodchild and Vilar-Alvarez (1962) reported that cysticercoids did not develop in rats in which the anterior one-third of the small intestine was removed and the intestine was reconstituted with the bile duct cannulated to the caecum. On the other hand, when the anterior one-third of the small intestine was removed posterior to the

bile and pancreatic duct attachments 70% of the cysticercoids fed to each rat developed to maturity. Development of the tapeworms also occurred when the posterior one-third or the posterior one-half of the small intestine was removed, with a recovery of 67 and 63% respectively of 30 larvae in 10 rats in each case. They suggested that a lack of bile has a greater depressing effect on the maturation of H. diminuta than does the shortening of the rat's small intestine. Further, Bemrick (1963) reported that 97% of 51 rats surgically altered to prevent bile flow lost previous infections of Giardia duodenalis.

Clearly the normal flow of bile is advantageous to the establishment, growth and development of the larval forms and to the maintenance of adult cestodes in the normal definitive host.

In view of the aforementioned reports on amino acid metabolism in cestodes and the effects of bile and bile salts on their metabolism, I designed a study to test the relative absorption of amino acids by cestodes from, and in the presence or absence of, diluted secreted bile. Experiments were designed to determine: 1) the effect of host bile on amino acid absorption in vitro, 2) the effect of the absence of bile on amino acid absorption in vivo, and 3) the fate of the labeled amino acids absorbed in vitro and in vivo. In an effort to obtain some idea of the general application of the results, comparative measurements were obtained using two closely related cestodes, H. diminuta and H. megalocephala, both of which thrive in the same host species.

CHAPTER II

MATERIALS AND METHODS

Biological

Cotton Rats

Laboratory reared cotton rats (Sigmodon hispidus Say and Ord, 1825) served as the host animal in this investigation. They descended from a colony of wild cotton rats live-trapped near the University of Oklahoma. The colonized laboratory animals reproduced while housed in modified rat cages for four years under relatively standard conditions. They were fed a standard rat chow for ad libitum ingestion supplemented with lettuce leaves and occasionally with wheat germ oil.

Beetles

A small colony of Tribolium confusum Duval, 1868, was isolated from a sack of commercial rat chow and perpetuated for use as the intermediate host for all larval cestode infections.

Cestodes

The experimental cestodes used in this work were Hymenolepis diminuta (Rudolphi, 1819) and Hymenolepis megalocephala, sp. n. All H. diminuta employed were propagated from a natural infection in a wild caught Rattus norvegicus (Berkenhout, 1769).

A young male cotton rat, trapped at Etowah, Cleveland County, Oklahoma, in 1962, was infected with H. megalocephala, sp. n.,

(description in manuscript), and all experimental worms were propagated from it.

Both H. diminuta and H. megalocephala become patent adults in cotton rats in 14 days, and the cysticercoids of each become infective for cotton rats 14 days after egg ingestion by T. confusum maintained at room temperature.

Experimental Infections

Young adult T. confusum, deprived of food for at least 4 days, were exposed to fresh apple pulp with which cestode eggs from freshly passed proglottids were thoroughly mixed. After feeding on this mixture, the beetles were maintained on an appropriate diet at normal room temperature. Cysticercoid infections were readily achieved for both cestodes and infections were so scheduled that larvae of at least 14 days of age were routinely available.

Groups of 8 adult virgin female cotton rats, starved 6 to 24 hours, were infected with cysticercoids by stomach tube. Initially 4 and later 3 cysticercoids were given to each rat.

In Vitro Experiments

Infected rats were killed by a blow on the head, mainly 14 or 15 days after infection, and the small intestines quickly excised. Cestodes were flushed from the intestine with a balanced salt solution employed by Read, et al., (1963). The worms were rinsed free of debris, randomized relative to the individual hosts, blotted free of excess saline, and the wet weight determined with a Roller-Smith balance. After wet weight determination, the worms were preincubated aerobically in 4 ml. of balanced saline at 37 C. for 60 to 90 minutes.

Following preincubation, the worms were blotted and transferred

to 25 ml. Erlenmeyer flasks containing 4 ml. of balanced saline with radioactive glycine. Fifty percent of the flasks also contained 10% host gall bladder bile, which approximates normal bile concentration in the small intestine (Smyth and Haslewood 1963). Each flask was maintained under air at 37 C. for 60 to 120 minutes in a water bath with continual shaking. The total radioactivity of the Glycine-C^{14} in each flask was 33.3 microcuries, but the molar concentration varied with the label position in the three different samples used. The mM concentration of the labeled glycine stock solutions was not adjusted to equality by the addition of nonlabeled glycine in an effort to promote a greater absorption of the labeled molecular species.

The six different types of experiments were as follows:

Expt. No.

1. Glycine-1-C^{14} (7.26 mMoles at 1.81 mM final conc.)
2. Glycine-1-C^{14} (7.26 mMoles + 10% bile)
3. Glycine-2-C^{14} (10.76 mMoles at 2.69 mM final conc.)
4. Glycine-2-C^{14} (10.76 mMoles + 10% bile)
5. Glycine-UL-C^{14} (0.395 mMoles at 0.099 mM final conc.)
6. Glycine-UL-C^{14} (0.395 mMoles + 10% bile)

At the end of the incubation period the worms were rinsed in 4 separate 50 ml. volumes of balanced saline to remove unincorporated Glycine-C^{14}. The worms were placed in 2 ml. of 80 to 100% ethanol and stored in 15 ml. screw cap culture tubes at -5 C. until fractionation analyses were carried out. Incubation media, containing the unabsorbed Glycine-C^{14}, was preserved in an appropriate volume of ethanol and stored until total radioactivity was determined.

Experiments with H. diminuta were carried out with one worm per

incubation flask. Experiments with H. megalocephala were carried out with two worms per incubation flask.

In Vivo Experiments

In the in vivo experiments, each of 6 infected virgin female rats were given 1 ml. of the stock Glycine-C^{14} solution containing 33.3 microcuries per ml. Administration was by stomach tube or intraperitoneal injection as follows:

Expt. No.	Molecular species and Route of Administration
1.	Glycine-1-C^{14} (7.26 mMoles) stomach tube
2.	Glycine-2-C^{14} (10.76 mMoles) stomach tube
3.	Glycine-UL-C^{14} (0.395 mMoles) stomach tube
4.	Glycine-1-C^{14} (7.26 mMoles) I. P. injection
5.	Glycine-2-C^{14} (10.76 mMoles) I. P. injection
6.	Glycine-UL-C^{14} (0.395 mMoles) I. P. injection

The same method was used for administration to 6 rats whose common bile ducts had been ligated in three places 24 hours prior to administration.

The above twelve types of in vivo experiments were scheduled for both H. diminuta and H. megalocephala.

After an interval of absorption time the hosts were killed, the cestodes were flushed quickly from the intestines and rapidly rinsed in 3 to 4 separate 50 ml. volumes of balanced saline. They were then blotted, weighed, and placed in culture tubes with 2 ml. of 100% ethanol. The tubes were stored at -5 C. until fractionation analyses. The intestinal mucosa of each rat was scraped from the first 10 inches of the small intestine and stored in 100% ethanol for future determination of absorbed C^{14}. Bile from each gall bladder was measured

and introduced into 1 ml. of 100% ethanol and stored in a similar fashion.

In the experiments in which the labeled glycine was given by stomach tube, one to two hours absorption time was allowed prior to killing the hosts, and four hours elapsed in the case of the intraperitoneally injected animals.

Biochemical

Free amino acids, lipids, glycogen, and protein were isolated from experimental tissue by a scheme modified from Hopkins (1960). Tissues from each experiment were extracted individually and Glycine-C^{14}, or the radioactivity therefrom, incorporated into each biochemical fraction was determined by measuring the radioactivity.

The tissue and its preservative were transferred into a 15 ml. homogenizer, adjusted to 5 ml. with 80% ethanol, homogenized, and then heated in a boiling water bath to precipitate coagulable proteins. After cooling, the mixture was homogenized again and aliquots taken for determination of total radioactivity. The remainder was centrifuged at 1,000 x g for 10 minutes, the ethanolic supernatant decanted and saved. The precipitate was washed twice with 1 ml. of 80% ethanol and saved. The washings were added to the supernatant. Three volumes of chloroform were added to the supernatant and washings and the two solutions mixed by shaking. Upon standing the mixture separated into an aqueous and a chloroform phase. The upper aqueous phase containing the free amino acids was transferred to calibrated glassware and adjusted to a known volume and aliquots taken for determination of radioactivity. The chloroform phase was saved for further fractionation.

The precipitate was extracted with 4 volumes (w/v) of chloroform: methanol (2:1, v/v) to remove lipids. The extract was combined with the chloroform fraction from the supernatant. This mixture was washed with water to remove nonlipid contamination and the chloroform evaporated under a stream of air at 60 C. The total lipid residue was dissolved in a known volume of chloroform and aliquots taken for determination of radioactivity.

The lipid extracted precipitate was dissolved in 40% KOH and digested overnight in a waterbath at 60 C. Glycogen was precipitated from the digest with ethanol. The solution was centrifuged and the supernatant decanted and adjusted to a known volume. Aliquots of the supernatant containing the protein fraction were taken for determination of radioactivity. Precipitated glycogen was washed twice with 60% ethanol, dissolved in water, adjusted to a known volume, and aliquots taken for radioactivity determination.

Host Mucosa

Ethanol was evaporated under air at 60 C. from the mucosa samples and the residue digested in 40% KOH and adjusted to a known volume for determination of radioactivity.

In Vivo Host Bile

Ethanol was evaporated as above from the bile samples collected in the in vivo experiments. The residue was dissolved in glass distilled water and adjusted to a known volume for determination of radioactivity.

Radioisotopic

All planchets were counted for 10 minutes or until 10^6 counts were recorded. Counts were made in a Nuclear Chicago Model D47 Gas

Flow Detector, with a thin window GM tube, assembled in a Model C110B Automatic Sample Changer, and used with a Model 8703 Scaler with Lister. Q-gas, 99.05% Helium with 0.95% Isobutane, was used in the detector system. The efficiency of the counting system was 37% as determined by a standard C^{14} sample.

CHAPTER III

RESULTS

Infected Hosts: Weight Relationships and Bile

The cotton rats infected with H. diminuta and H. megalocephala weighed between 127 and 250 grams. One group of hosts infected with the latter cestode showed a slight gain in weight during infection. All other hosts lost weight while infected. There was no apparent correlation between the number of cestodes or their total weight and the weight change in the hosts.

In the in vivo work, some gall bladders were ruptured and part or all of the bile lost. Bladders of infected hosts from which all the bile was collected contained 20 to 50 microliters, except for one which contained 100 microliters. Bladders of hosts whose bile ducts were ligated for 24 hours before experimentation contained 75 to 100 microliters of bile, except that one contained 150 microliters.

During preliminary infection experiments of 2 years duration 75 to 100% recovery of adult cestodes from the administration of three or four cysticercoids was routinely attained, but such was not the case in the glycine absorption work reported here. Some rats did not become infected at all. Moreover, some animals which became infected did not retain the worms nearly as well as did those in the preliminary experiments, in which both species of worms were retained up to 90 days.

The percent recovery of adults from experimentally infected cotton rats given 4 cysticercoids of either species varied from 25 to 100% per rat, and averaged 50% and 62.5% for H. diminuta and H. megalocephala, respectively. In both H. diminuta and H. megalocephala infections of three cysticercoids, the percent recovery of adults averaged 66.6%, with a range of 33.3 to 100% per rat. Those animals whose bile ducts were ligated tended to lose their infections during the following 25 hours, and the percent recovery of worms was lowest in this experimental group at 33.3%.

Cestodes

Fourteen day old adult H. diminuta from cotton rats averaged 208.3 milligrams fresh wet weight, while 15 day old H. megalocephala averaged 173.5 mg. Both of these values were lowered considerably by a small number of extremely small worms. Twenty to 25 day old adult H. diminuta averaged 434 mg. fresh wet weight.

H. diminuta showed a dry weight of 18.5%, while H. megalocephala had a dry weight of 18.3%.

Glycine-C^{14} Absorption

Tables 1, 3, 5, and 7 show the total counts per minute of C^{14} radioactivity per 100 mg of tissue per hour and the percent incorporation of the available radioactive glycine (Table 7 also shows the count per inch of mucosa and per microliter of bile). Tables 2, 4, 6, and 9 show the total micromoles of radioactive glycine absorbed or incorporated per 100 mg of cestode tissue per hour and the percentage distribution of the absorbed radioactivity into the 4 biochemical fractions. Table 8 shows the total micromolar absorption in the cestode free amino acid fractions compared to that in the mucosa and bile

of individual hosts. The value listed below each entry in Tables 1, 3, 5, 7, and 8 is the percentage of total available radioactivity absorbed or incorporated. Further, the percentages in these tables pertain to total absorption, by all tissue present, during the entire incubation or absorption period. They are not reported on a per 100 mg of tissue per hour basis. The value listed below each entry of Tables 2, 4, 6, and 9 is the percentage of total absorption detected in the particular biochemical fraction or tissue, unless otherwise stated.

In Vitro

In vitro absorption of Glycine-C^{14} by H. diminuta and H. megalocephala (Tables 1, 2, and 9) was quite pronounced even though some differences between individual experiments occurred. The total count per minute was slightly lower in the whole homogenate than in the free amino acid fraction in 3 of the 15 experiments. This indicates slight self-absorption in plated out samples or slightly heterogeneous distribution of the labeled glycine in the whole homogenate.

In the in vitro experiments each species of cestode was incubated individually in Glycine-1-C^{14}, Glycine-2-C^{14}, and Glycine-UL-C^{14}. In each case separate worm samples were incubated in the presence, and absence, of host bile (Tables 1, 2, and 9). By summing the C^{14} found in the 4 biochemical fractions reported in Table 1 it is possible to gain a rough measure of total glycine incorporation in each experiment. When this is done, and all incubations are considered, H. diminuta is found to incorporate 24.5% of the total available glycine when bile is omitted. H. megalocephala incorporated 35.3% of the total available glycine. With bile present in the incubation medium H. diminuta incorporated 10.3% of the available glycine, while H. megalocephala

incorporated 14.5% of this amino acid. It is obvious that under these experimental conditions Glycine-C^{14} incorporation is inhibited when 10% v/v gall bladder bile is a component of the incubation medium. This effect is also shown in Tables 2 and 9.

Although some variation between individual experiments occurred, neither the label position of the Glycine-C^{14} sample used nor the effect of incubation in the presence of bile had a significant effect on the distribution of the absorbed glycine into the 4 major biochemical fractions of either species of cestode (Tables 1 and 2). The free amino acid fraction of H. diminuta contained 93 to 97% of the absorbed glycine, while this fraction of H. megalocephala contained 92 to 95% of the glycine (Table 2). Up to 6% of the absorbed glycine was incorporated into the protein fractions of both species of cestode. The greatest incorporation of absorbed radioactivity into the lipid fraction was 1.66% for H. diminuta and 1.91% for H. megalocephala. H. diminuta incorporated a maximum of 0.1% and H. megalocephala a maximum of 0.014% of the absorbed radioactivity into the glycogen fraction.

H. megalocephala was apparently much more efficient in glycine absorption under the conditions of the in vitro incubations than was H. diminuta. However, in 2 of the 15 experiments (Glycine-2-C^{14} with bile and Glycine-UL-C^{14} without bile) the latter cestode absorbed more glycine than did the former (Tables 2 and 9). The inhibitory effect of bile was greatest in H. megalocephala, yet in one of the six experiments (Glycine-UL-C^{14}) this effect appears to be greatest in H. diminuta (Table 9).

In these studies no evidence was found for the preferential decarboxylation of any of the differently labeled molecular species

of glycine. Further, there was no evidence that either molecular species was metabolized in a different manner by either cestode.

In Vivo

In the in vivo studies the hosts absorbed most of the Glycine-C^{14}, and consequently the absorption by the cestodes was very low compared to absorption by cestodes in vitro (Table 9). The whole homogenate counts were again slightly lower than those of the free amino acid fraction in 6 of the 16 in vivo experiments. This indicates slight self-absorption, or unequal distribution of the isotope-labeled glycine in the whole homogenate. The radioactivity in the mucosa and bile at the time of autopsy was relatively high in most of the experiments.

Glycine Absorption in Normal Hosts: In the stomach tube administrations, H. diminuta absorbed 1.06% of the total dose of Glycine-1-C^{14}, 0.76% of the Glycine-2-C^{14}, and 0.01% of the Glycine-UL-C^{14}. In the experiment with UL-C^{14} the cotton rat regurgitated most of the glycine. In comparison, H. megalocephala absorbed 0.26% of the dose of Glycine-1-C^{14}, 0.65% of the Glycine-2-C^{14}, and 0.08% of the Glycine-UL-C^{14}. When the glycine was administered intraperitoneally, H. diminuta absorbed a total of 0.14% of the dose of Glycine-1-C^{14}, 0.75% of the Glycine-2-C^{14}, and 0.19% of the Glycine-UL-C^{14}. In comparison, H. megalocephala absorbed 0.07% of the Glycine-1-C^{14}, 0.17% of the Glycine-2-C^{14}, and 0.17% of the Glycine-UL-C^{14} (Table 3).

The percentage distribution of the absorbed Glycine-C^{14} is somewhat different in the in vivo experiments and in the in vitro studies. This is more apparent when one compares the biochemical distribution presented in Tables 2 and 4.

In the stomach tube administrations, excepting the Glycine-UL-C^{14}

experiments, H. diminuta retained 87 to 90% and H. megalocephala retained 91 to 92% of the glycine in the free amino acid fraction. In the intraperitoneal administrations, on the other hand, H. diminuta retained 70 to 88% and H. megalocephala retained 82 to 91% in this fraction (Table 4). H. diminuta incorporated up to 12% and H. megalocephala up to 8% of the absorbed glycine into the protein fraction, and they incorporated 29% and 15% respectively when administration was intraperitoneal. H. diminuta incorporated a maximum of 0.06% and H. megalocephala incorporated 0.77% of the absorbed radioactivity into the lipid fraction in the stomach tube administration, and 0.35% and 0.84% respectively when administration was intraperitoneal. The incorporation of the absorbed radioactivity into the glycogen fraction was 0.12% for H. diminuta and 0.6% for H. megalocephala in the stomach tube administrations, and 0.59% and 1.72% respectively when administration was intraperitoneal (Table 4).

The results of the Glycine-UL-C^{14} experiment with H. megalocephala in the stomach tube administrations, shown in Table 4, are not included in the results above, because of the relatively great variation from the results of the other experiments of this group. These results (Table 4) clearly show that a greater distribution into the lipid and glycogen fractions occurs in H. megalocephala than in H. diminuta, but greater incorporation into protein occurs in the latter.

A comparison of the absorption by the host mucosa to that into the cestode free amino acid fractions (Tables 7 and 8) shows that the mucosa contained considerably more radioactivity at autopsy than did the cestodes, regardless of the route of administration of the labeled glycine. In all intraperitoneal injections in which bile

samples were collected, the secretion of radioactive material into the bile was greater than when administration was by stomach tube (Tables 7 and 8). The total incorporation of radioactivity into the bile was comparable to that in the cestodes in the H. megalocephala experiments in which Glycine-2-C^{14} was given intraperitoneally. In the Glycine-1-C^{14} intraperitoneal injections, the gall bladders were ruptured during autopsy and the bile was lost.

Glycine Absorption in Bile Duct Ligated Hosts: At the time of these experiments, infecting the cotton rats with either species of cestode became increasingly and unexplainably difficult. Some of the animals infected at the time of bile duct ligation did not retain the cestodes for 25 hours thereafter. Because of these difficulties, fewer experiments on Glycine-C^{14} absorption in this group of experimental animals were completed.

Twenty-five hours after ligation of the common bile duct, no whole living H. diminuta was recovered from the hosts. However, one rat retained a piece of the anterior part of a strobila without the scolex, in the small intestine, and many short motile pieces of more mature regions of the strobila in the caecum. Three hosts retained H. megalocephala 25 to 26 hours after ligation of the bile duct; one host, infected at surgery, was not infected at autopsy.

In these experiments all Glycine-C^{14} administrations to bile duct ligated hosts were by stomach tube. In the experiment using Glycine-UL-C^{14} no cestode was recovered.

In the Glycine-1-C^{14} experiments, 66 mg of H. diminuta from the small intestine absorbed 0.01% of the dose, while 509 mg of the same worm(s) from the caecum absorbed 0.01%. H. megalocephala absorbed

0.01% of the Glycine-1-C^{14} (Table 5). In the Glycine-2-C^{14} experiments, H. megalocephala absorbed 0.02% in one experiment, and 0.85% in another experiment (Table 5). The micromolar absorption is presented in Tables 6 and 9.

The percentage distribution of the absorbed radioactivity into the 4 biochemical fractions was quite different in these bile duct ligated host experiments from that in the normal host experiments, as is seen by comparing Tables 4 and 6.

In the stomach tube administered Glycine-1-C^{14} experiments, 66 mg of H. diminuta tissue from the small intestine retained 60.3% of the absorbed glycine in the free amino acid fraction, while 509 mg of tissue of the same worm(s) from the caecum retained only 41.3% of the glycine in this fraction. H. megalocephala retained 66.6% of the absorbed glycine in the free amino acid fraction. The incorporation into protein was 38.1% by the 66 mg of H. diminuta tissue, and 53.4% by the 509 mg of tissue, while H. megalocephala incorporated 31% of the absorbed glycine into the protein fraction (Table 6). The 66 mg of H. diminuta incorporated 1.57%, and the 509 mg incorporated 0.86% of the absorbed radioactivity into the lipid fraction. H. megalocephala incorporated 0.57% of the absorbed radioactivity into the lipid fraction. The incorporation of absorbed radioactivity into glycogen was not significantly above background radiation to be determined for the 66 mg of H. diminuta tissue; however, the 509 mg of tissue incorporated 4.31% of the absorbed radioactivity into the glycogen fraction. H. megalocephala incorporated 1.7% of the absorbed radioactivity into the glycogen fraction (Table 6).

In the Glycine-2-C^{14} administrations, H. megalocephala retained

68.8% of the absorbed glycine in the free amino acid fraction, and 95.2% in a repeat experiment. It incorporated 25.4%, and 3.7% in a repeat, of the absorbed glycine into the protein fraction. The incorporation of radioactivity into the lipid fraction by H. megalocephala was 0.32% and 0.91% in a repeat. This cestode incorporated 5.3%, and 0.11% in a repeat, of the absorbed radioactivity into the glycogen fraction (Table 6).

The absorption of radioactivity by the mucosa (Tables 7 and 8) was much greater than that of the cestodes except in the repeat experiment with H. megalocephala in which Glycine-2-C^{14} was administered, and in which the absorption by the host mucosa was only about one-seventh that of the cestode. In the experiment with Glycine-2-C^{14} the host incorporated more radioactivity into the bile than was absorbed by H. megalocephala, although in the other experiments of this group the bile contained somewhat less radioactivity than did the cestodes (Table 8). The data presented in this table give the overall picture of the absorption in the cestodes compared to that in the mucosa and bile of individual hosts.

TABLE 1

IN VITRO GLYCINE-C^{14} ABSORPTION BY H. DIMINUTA AND H. MEGALOCEPHALA

		Free A As		Total Lipids		Glycogen		Protein		In Medium	
		wo bile	w bile	wo bile	w bile	wo bile	w bile	wo bile	w bile	wo bile	w bile
1-C^{14}	Hd	6392.15	5263.55	113.33	75.04	6.95	2.64	319.42	218.35	37394.30	85845.00
	%	25.27	10.33	0.45	0.15	0.03	0.01	1.26	0.43	47.84	68.40
	Hd	12244.43	---	58.18	---	2.40	---	249.15	---	86839.30	---
	%	18.60	---	0.09	---	0.00	---	0.38	---	48.89	---
	Hm	16244.30	8054.00	128.21	70.71	1.85	1.25	630.65	360.43	28945.60	35239.70
	%	27.09	14.37	0.21	0.13	0.00	0.00	1.05	0.64	48.26	62.88
2-C^{14}	Hd	8419.85	6517.10	142.78	108.79	1.37	1.06	235.44	277.14	98436.50	112213.60
	%	13.85	10.25	0.23	0.17	0.00	0.00	0.39	0.44	61.89	76.53
	Hd	7486.89	---	68.66	---	2.72	---	252.68	---	114322.50	---
	%	9.21	---	0.08	---	0.00	---	0.31	---	57.62	---
	Hm	14866.60	4129.70	140.56	43.84	1.70	0.51	765.47	154.45	27661.70	46826.00
	%	26.47	6.30	0.25	0.07	0.00	0.00	1.36	0.24	49.25	71.49
ULC^{14}	Hd	30858.85	9909.40	323.32	101.14	7.18	3.02	1029.28	550.16	127207.50	207944.80
	%	34.74	8.64	0.36	0.09	0.01	0.00	1.16	0.48	43.22	81.24
	Hd	14545.63	---	15.61	---	5.88	---	976.31	---	7800.27	---
	%	37.82	---	0.04	---	0.02	---	2.51	---	20.28	---
	Hm	24661.30	10591.80	511.75	137.96	3.99	2.62	1523.60	692.65	9769.20	31058.50
	%	45.68	20.34	0.95	0.26	0.01	0.01	2.82	1.33	18.09	59.64

Total cpm x 10^2/100 mgm Tissue per Hour, and Percent incorporation of available radioactive carbon. wo bile = without bile, w bile = w bile In Medium = the cpm remaining in the medium are reported per 100 mgm cestode tissue incubated therein per hour. Hd = H. diminuta Hm = H. megalocephala

TABLE 2

IN VITRO GLYCINE-C^{14} ABSORPTION BY H. DIMINUTA AND H. MEGALOCEPHALA

		Free As A		Total Lipids		Glocogen		Protein	
		wo bile	w bile	wo bile	w bile	wo bile	w bile	wo bile	w bile
1-C^{14}	Hd	175.07	144.04	3.10	2.05	0.19	0.07	8.75	5.98
	%	93.56	94.67	1.66	1.35	0.10	0.05	4.67	3.93
	Hd	334.71	---	1.99	--	0.06	--	6.81	--
	%	97.53	---	0.46	--	0.02	--	1.98	--
	Hm	444.90	220.58	3.51	1.94	0.05	0.03	17.27	9.87
	%	95.53	94.91	0.75	0.83	0.01	0.01	3.71	4.25
2-C^{14}	Hd	323.91	250.68	5.49	4.18	0.05	0.04	9.06	10.66
	%	95.69	94.40	1.62	1.58	0.02	0.01	2.68	4.01
	Hd	287.23	---	2.63	--	0.10	--	9.70	--
	%	95.85	---	0.88	--	0.03	--	3.24	--
	Hm	571.92	158.87	5.41	1.69	0.06	0.02	29.45	5.94
	%	94.25	95.41	0.89	1.01	0.01	0.01	4.85	3.57
UL-C^{14}	Hd	55.40	17.76	0.58	0.18	0.01	0.01	1.85	0.98
	%	95.79	93.84	1.00	0.94	0.02	0.03	3.19	5.20
	Hd	26.07	---	0.03	--	0.01	--	1.03	--
	%	93.64	---	0.10	--	0.04	--	6.22	--
	Hm	44.29	19.02	0.92	0.25	0.00	0.00	2.74	1.24
	%	92.38	92.74	1.91	1.19	0.01	0.01	5.70	6.06

Total Micromoles Incorporated/100 mgm Tissue per Hour, and Percent of Total Absorbed Radioactivity in each Biochemical Fraction.

Hd = H. diminuta Hm = H. megalocephala

TABLE 3

IN VIVO GLYCINE-C^{14} ABSORPTION BY H. DIMINUTA AND H. MEGALOCEPHALA IN NORMAL HOSTS

		Free A As		Total Lipids		Glycogen		Protein	
		St t	I P	St t	I P	St t	I P	St t	I P
1-C^{14}	Hd	150.76	10.92	0.06	0.04	0.11	0.09	16.18	1.26
	%	0.95	0.13	0.00	0.00	0.00	0.00	0.10	0.01
	Hm	116.67	17.42	1.04	0.14	0.77	0.36	8.28	3.31
	%	0.24	0.06	0.00	0.00	0.00	0.00	0.02	0.01
2-C^{14}	Hd	110.45	57.34	0.11	0.27	0.19	0.14	15.77	23.87
	%	0.66	0.53	0.00	0.00	0.00	0.00	0.09	0.22
	Hm	848.68	46.46	2.98	0.29	2.85	8.43	75.42	3.14
	%	0.59	0.14	0.00	0.00	0.00	0.02	0.05	0.01
UL-C^{14}	Hd	1.63*	9.38	0.01	0.06	---	0.06	1.42	13.78
	%	0.01	0.16	---	0.00	---	--	0.01	0.03
	Hm	17.49	13.72	0.20	0.19	0.61	0.15	3.93	2.31
	%	0.06	0.14	0.00	0.00	0.00	0.00	0.01	0.02

Total cpm x 10^2/100 mgm Tissue per Hour, and Percent Incorporation of Total Radioactivity Administered.

*this rat regurgitated some of the glycine; it is estimated that 0.4 of 1.0 ml remained in the stomach.

St t = stomach tube, I P = intraperitoneal injection

Hd = H. diminuta Hm = H. megalocephala

TABLE 4

IN VIVO GLYCINE-C^{14} ABSORPTION BY H. DIMINUTA AND H. MEGALOCEPHALA IN NORMAL HOSTS

		Free A As		Total Lipids		Glycogen		Protein	
		St t	I P	St t	I P	St t	I P	St t	I P
1-C^{14}	Hd	4.13	0.30	0.00	0.00	0.00	0.00	0.44	0.04
	%	90.23	88.72	0.03	0.30	0.07	0.59	9.68	10.39
	Hm	3.19	0.48	0.03	0.00	0.02	0.01	0.23	0.09
	%	92.10	82.07	0.78	0.69	0.61	1.72	6.52	15.52
2-C^{14}	Hd	4.25	2.21	0.00	0.01	0.01	0.01	0.60	0.92
	%	87.45	70.25	0.06	0.35	0.12	0.16	12.37	29.27
	Hm	32.46	1.79	0.11	0.01	0.09	0.03	2.88	0.12
	%	91.33	91.62	0.31	0.56	0.24	1.66	8.11	6.16
UL-C^{14}	Hd	0.00*	0.02	--	--	--	--	0.00*	0.00
	%	56.52	85.00	--	--	--	--	43.48	15.00
	Hm	0.03	0.03	0.00	0.00	0.00	0.00	0.01	0.00
	%	76.92	84.75	5.13	0.85	2.56	0.85	15.38	13.56

Total Micromoles Incorporated/100 mgm Tissue per Hour , and Percent of Total Absorbed Radioactivity in Each Biochemical Fraction.

*this rat regurgitated some of the glycine; it is estimated that 0.4 of the 1.0 ml remained in the stomach.

Hd = H. diminuta Hm = H. megalocephala

TABLE 5

IN VIVO GLYCINE-C^{14} ABSORPTION BY H. DIMINUTA AND H. MEGALOCEPHALA IN BILE DUCT LIGATED HOSTS

		Free A As St t	Total Lipids St t	Glycogen St t	Protein St t
1-C^{14}	Hd	11.27*	0.33	--	7.14
	%	0.00	0.00	--	0.00
	Hd	1.78**	0.05	0.19	2.29
	%	0.00	0.00	0.00	0.01
	Hm	8.56	0.09	0.22	3.20
	%	0.01	--	0.00	0.00
2-C^{14}	Hm	11.04	0.07	0.86	4.11
	%	0.01	--	0.00	0.01
	Hm	364.00***	3.49	0.45	14.22
	%	0.80	0.01	0.00	0.03

Total cpm x 10^2/100 mgm Tissue per Hour, and Percent Incorporation of Total Radioactivity Administered.

*66 mgm cestode tissue from small intestine

**509 mgm cestode tissue from caecum

***a duplicate experiment

Hd = H. diminuta Hm = H. megalocephala

TABLE 6

IN VIVO GLYCINE-C^{14} ABSORPTION BY H. DIMINUTA AND H. MEGALOCEPHALA IN BILE DUCT LIGATED HOSTS

		Free A As St t	Total Lipids St t	Glycogen St t	Protein St t
1-C^{14}	Hd	0.31*	0.01	--	0.19
	%	60.31	1.57	--	38.11
	Hd	0.05**	0.00	0.01	0.06
	%	41.38	0.86	4.31	53.45
	Hm	0.23	0.00	0.01	0.11
	%	66.67	0.57	1.71	31.05
2-C^{14}	Hm	0.42	0.00	0.03	0.16
	%	68.83	0.32	5.36	25.49
	Hm	14.00***	0.13	0.02	0.55
	%	95.26	0.91	0.11	3.72

Total Micromoles Incorporated/100 mgm Tissue per Hour, and Percent of Total Absorbed Radioactivity in Each Biochemical Fraction.

*66 mgm cestode tissue from small intestine

**509 mgm cestode tissue from caecum

***a duplicate experiment

No cestodes were recovered in the Glycine-UL-C^{14} experiments

Hd = H. diminuta Hm = H. megalocephala

TABLE 7

IN VIVO GLYCINE-C^{14} ABSORPTION BY H. DIMINUTA AND H. MEGALOCEPHALA IN THE FREE AMINO ACID FRACTION IN NORMAL AND BILE DUCT LIGATED HOSTS, COMPARED TO THE ABSORPTION BY THE MUCOSA AND GALL BLADDER BILE OF THE HOSTS

		NORMAL HOSTS						BILE DUCT LIGATED HOSTS		
		Free A As		Mucosa		Bile		Free A As	Mucosa	Bile
		St t	I P	St t	I P	St t	I P	St t	St t	St t
1-C^{14}	Hd	150.76	10.92	186.36	18.66	1.15	---	11.27*	630.89	0.07
	%	0.95	0.13	1.05	0.28	0.01	---	0.00	2.97	0.00
								1.78**		
								0.00		
	Hm	116.67	17.42	437.88	36.63	2.83	---	8.56	243.45	0.16
	%	0.24	0.06	2.06	0.55	0.06	---	0.01	1.15	0.01
2-C^{14}	Hd	110.45	57.34	250.23	109.76	2.30	1.85	---	---	---
	%	0.66	0.53	1.34	1.57	0.03	0.07	---	---	---
	Hm	848.68	46.46	616.72	48.91	15.95	2.41	1.04	55.57	1.07
	%	0.59	0.14	2.56	0.70	0.10	0.10	0.01	0.30	0.04
								364.00***	33.16	---
								0.80	0.12	---
UL-C^{14}	Hd	1.63+	9.38	13.77	33.99	0.11	0.51	---	---	---
	%	0.01	0.16	0.09	0.62	0.00	0.09	---	---	---
	Hm	17.49	13.72	229.31	20.32	1.64	3.04	---	500.12	0.15
	%	0.06	0.14	1.30	0.37	0.05	0.02	---	9.97	0.01

Total cpm x 10^2/100 mgm Tissue per Hour,/Inch Mucosa per Hour, and/Microliter of Bile per Hour; and Percent Incorporation of Total Radioactivity Administered. *66 mgm cestode tissue from small intestine; **509 mgm cestode tissue from caecum; *** a duplicate experiment; +regurgitated retained est. 0.4 of 1.0 ml in stomach. Hd = H. diminuta Hm = H. megalocephala

TABLE 8

IN VIVO GLYCINE-C^{14} ABSORPTION BY H. DIMINUTA AND H. MEGALOCEPHALA IN THE FREE AMINO ACID FRACTION IN NORMAL AND BILE DUCT LIGATED HOSTS, COMPARED TO THE ABSORPTION BY THE MUCOSA AND GALL BLADDER BILE OF THE HOSTS

		NORMAL HOSTS						BILE DUCT LIGATED HOSTS		
		Free A As		Mucosa		Bile		Free A As	Mucosa	Bile
		St t	I P	St t	I P	St t	I P	St t	St t	St t
1-C^{14}	Hd	69.31	9.16	76.56	20.45	0.94	---	0.56	215.98	0.36
	%	0.95	0.13	1.05	0.28	0.01	---	0.01	2.97	0.01
	Hm	17.53	4.61	149.91	40.13	4.37	---	0.59	83.34	0.48
	%	0.24	0.06	2.06	0.55	0.06	---	0.01	1.15	0.01
2-C^{14}	Hd	71.38	56.73	144.40	168.90	3.19	7.12			
	%	0.66	0.53	1.34	1.57	0.03	0.06			
	Hm	63.62	14.72	275.21	75.27	10.67	10.93	1.47	32.07	4.62
	%	0.59	0.14	2.56	0.70	0.10	0.10	0.01	0.30	0.43
	Hm							85.56	12.70	---
	%							0.80	0.12	---
UL-C^{14}	Hd	0.03	0.65	0.37	2.44	0.01	0.37	---	---	---
	%	0.01	0.16	0.09	0.62	0.00	0.09	---	---	---
	Hm	0.24	0.57	5.15	1.46	0.03	0.09	---	15.72	0.05
	%	0.06	0.14	1.30	0.37	0.05	0.02	---	3.97	0.01

Total Micromoles Incorporated, and Percent Incorporated of Total Radioactivity Administered.

Hd = H. diminuta Hm = H. megalocephala

TABLE 9

GLYCINE-C^{14} ABSORPTION BY H. DIMINUTA AND H. MEGALOCEPHALA

		In Vitro wo bile	In Vitro w bile	In Vivo Normal Hosts St t	In Vivo Normal Hosts I P	In Vivo B-D L Hosts St t
1-C^{14}	Hd	187.11	152.14	4.58	0.34	0.51*
	%		81.31		7.36	
	Hd	343.17	---	---	---	0.12**
	Hm	465.73	232.42	3.47	0.58	0.35
	%		49.91		16.72	
2-C^{14}	Hd	338.51	265.57	4.86	3.14	---
	%		78.45		64.62	
	Hd	299.67	---	---	---	---
	Hm	606.84	166.51	35.54	1.95	0.62
	%		27.44		5.49	
	Hm	---	---	---	---	14.70***
UL-C^{14}	Hd	57.84	18.93	0.00+	0.02	---
	%		32.72		434.78	
	Hd	27.84	---	---	---	---
	Hm	47.94	20.51	0.04	0.03	---
	%		42.78		75.64	

Total Micromoles Incorporated/100 mgm Tissue per Hour, and Percent Incorporation in the Presence of Bile, or from Intraperitoneal Injection, of the Incorporation in the Absence of Bile, or from Stomach Tube Administration

*66 mgm cestode tissue from small intestine

**509 mgm cestode tissue from caecum

***a duplicate experiment

+regurgitation, estim. 0.4 of 1.0 ml remained in stomach

Hd = H. diminuta Hm = H. megalocephala

CHAPTER IV

DISCUSSION

Host-Parasite Relationships

H. diminuta thrives about as well in the cotton rat, Sigmodon hispidus, as does the natural parasite, H. megalocephala. Neither cestode produces an observable deleterious effect on the host, with the exception that in H. diminuta infections the weight gain is not as great as in H. megalocephala infections. In the preliminary experiments 14 day old adult H. diminuta from four worm infections in cotton rats were found to measure 32, 32, 46, and 47 cm in length, and to weigh 178, 180, 226, and 306 mg, respectively. H. megalocephala of the same age measured 23, 24, 26, and 29 cm in length and weighed 84, 86, 128, and 142 mg, respectively. Both species are long lived in cotton rats, and in single worm infections of 70 to 90 days the worms were 26 to 30 inches long.

The gall bladders of normal rats, in the H. diminuta infections, contained on the average three-fifths of the volume of bile as in animals with H. megalocephala infections. Those hosts infected with the former cestode were slightly older and larger than were those infected with the latter cestode, and consequently one would expect them to produce slightly more bile. I have no rational explanation for this difference in bile production by infected hosts.

I cannot explain the lowered percentage recovery of adult cestodes of both species from the hosts, in the in vitro absorption studies. The loss of the cestodes, especially H. diminuta, during the 25 hours after the bile duct ligations, on the other hand, is thought to be directly related to the lack of bile in the lumen of the small intestine. The loss of H. diminuta under these conditions is in accord with some of the results obtained in the bile deprivation studies by Goodchild (1958a, 1958b, 1960, 1961a, 1961b), Vilar-Alvarez and Goodchild (1961), and Goodchild and Vilar-Alvarez (1962). Cestodes were readily visible through the thin intestinal walls of the cotton rats at the time of the bile duct ligation surgery, yet 25 hours later no whole intact adult H. diminuta was recovered from the small intestine of any host in this group. The natural parasite H. megalocephala, presumably much better adapted to the host, withstood the deprivation of bile for the 25 hours better than did H. diminuta. A total of 5 H. megalocephala were recovered from 3 hosts.

Although intestinal emptying time, quality of diet, and length of the small intestine have been implicated in the host specificity of cestode infections, it is suggested that the biochemistry and quantity of the bile of a host may also be a controlling factor which determines the degree of host specificity (Smyth and Haslewood, 1963).

In this connection, the development of a given cestode species in different laboratory hosts, or the susceptibility of age, strain, or sex groups of the same host species to a given parasite, such as covered in the studies by Schiller (1959), Litchford (1963), and Dow and Jarrett (1960), are of signal interest, and results obtained in their studies could well reflect effects of differences in the biochemistry

of host bile, as was suggested by Litchford. Read and Phifer (1959) have shown the importance of the quantity of host dietary carbohydrate in the interactions of two species in infections of one worm of each species in the same individual host, and Goodchild and Dennis (1965) have demonstrated slight size, weight, and biochemical composition differences in the same species of cestode reared in hosts on diets which differed slightly in protein quality. It is suggested that the host bile, relative to bile acid composition and quantity, is at least a mediator of the continued development and maintenance of established adult forms of cestodes and is probably very much involved in studies such as those above.

Specific bile acids and their salts and derived products have been shown to be toxic to cestode larvae. According to Read (1950a), glycocholic acid is toxic to Taenia pisiformis, and Smyth and Haslewood (1963) demonstrated that deoxycholic acid and its conjugated salts cause lysis of Echinococcus granulosus protoscolices. Dogs are the definitive hosts for both species, and both of these bile acids are absent, at least in lytic concentrations, from dog bile. Smyth and Haslewood suggested that the biochemical composition of the bile, whether or not all components are host produced, may be of paramount importance in the determination of host specificity to cestode infections in nature.

In Vitro Glycine Absorption

Ten percent whole gall bladder bile from cotton rats strikingly inhibited the absorption of Glycine-C^{14} by H. diminuta and H. megalocephala, in aerobic in vitro incubations (Tables 1, 2, and 9). The variation in the amount of absorption and inhibition in the 3 different

C^{14} label position samples is thought to be due mainly to the concentration of glycine in those respective experiments. The greatest absorption of glycine occurred in the experiments in which it was present in greatest concentration, and inhibition was greatest in those experiments in which it was least concentrated.

Although kinetic formulations were not employed in this work, it is believed that absorption was near maximal, and that the cestodes approached glycine saturation in the experiments in which the label was present in the number 1 and 2 positions, but not in those in which the glycine was unifformly labeled. This view is supported by the fact that the glycine concentration in the 1 and 2 labeled sample experiments was 2 to 3 times that calculated by Read, et al., (1963) to give maximal absorption under similar conditions in their study of methionine absorption. They calculated that one-half maximal absorption in 1 hour would occur at an initial concentration of 0.8 mM. In the uniformly labeled sample experiments the glycine concentration was only one-eighth of this calculated optimal concentration. Apparent differences in absorption between worm samples differing in weight were more closely reconciled after conversion into absorption per 100 mg tissue per hour.

The exact nature of the inhibition of Glycine-C^{14} absorption by the diluted bile was not determined; however, such inhibition could be accounted for by either specific inhibitory activity by one or more of the bile acids, or the conjugated salts or derivatives therefrom, in which case the inhibition would be noncompetitive in nature; or inhibitory activity of the amino acid pool of the bile, in which case the inhibition would be competitive in nature.

Except for the experiment with Glycine-2-C^{14} in the presence of

bile, H. megalocephala absorbed and retained a greater percentage of the total available Glycine-C^{14} in the in vitro incubations than did H. diminuta. Moreover, at the higher concentrations of glycine, the former cestode shows greater efficiency of absorption and retention per mg weight of tissue than does the latter (Tables 1, 2, and 9). H. diminuta may be more efficient at very low concentrations of glycine. The inhibitory effect of bile on absorption was much greater in H. megalocephala than in H. diminuta; this effect was probably related to the glycine concentration in the radioactive samples relative to that in the amino acid pool of the bile (Tables 1, 2, and 9).

In Vivo Glycine Absorption

The cotton rats absorbed most of the Glycine-C^{14} in the in vivo experiments, and consequently the absorption by the cestodes was extremely low compared to the absorption in vitro. Moreover, considerably variation in the results occurred in these experiments. In normal hosts, H. diminuta absorbed more of the administered glycine than did H. megalocephala. Absorption was greater by both species when administration was by stomach tube rather than by intraperitoneal injection, which indicates direct absorption from the lumen contents is of greater importance in the economy of cestodes than absorption from the host or host secretions (Tables 3, 4, and 9). The limited recovery of cestodes in the bile duct ligation experiments precludes a comparison between species in these experiments. In completed experiments, absorption of glycine by cestodes in these hosts was drastically less than that in normal hosts (Tables 5, 6, 7, 8, and 9).

In all but one of the in vivo experiments the host mucosa absorbed a greater percentage of the glycine than did the cestodes, and in all

but one experiment the amount of glycine absorbed by the cestodes was greater than that concentrated into the bile. The two exceptions were in H. megalocephala infections of bile duct ligated hosts in which Glycine-2-C^{14} was administered by stomach tube. The absorption of either Glycine-1-C^{14} or Glycine-2-C^{14} into the bile of ligated hosts approached the level of absorption in the harbored cestodes. Mucosal absorption was greatest when the glycine was administered by stomach tube, while the incorporation into the bile was greatest when the administration was intraperitoneal (Tables 7 and 8).

The absorption by the intestinal mucosa as compared to that of parasites in the same hosts has been studied previously by other investigators. Chandler, et al., (1950) reported that H. diminuta and the host mucosa absorbed S^{35} labeled thiamin to about the same "low level concentration" from parenteral injections of hosts maintained on a thiamin free diet and given sulfasuxidine to inhibit synthesis of vitamins by gut bacteria. Read (1950b) reported maximum absorption of stomach tube administered inorganic P^{32} by rat mucosa in 70 minutes, while the maximum absorption by H. diminuta occurred in 85 minutes. When 0.5% glucose was included in the phosphate sample, maximal absorption by the mucosa required 90 minutes, and there was very little absorption by the cestodes. In intraperitoneal injections the mucosa reached peak absorption in 220 minutes, while absorption by the cestodes continued to increase very slowly with time. Edmonds (1965) reported similar absorption of P^{32} by both host mucosa and the acanthocephalan, Moniliformis dubious, in oesophageal tube administrations, but absorption was comparatively lower in worms in intraperitoneal administrations.

He reported maximal absorption of L-leucine-C^{14} at 120 minutes by both host mucosa and M. dubious when rats were given the leucine by oesophageal tube. When administration was by intraperitoneal injection maximal absorption by the gut mucosa was at 1 hour, and that by M. dubious was at 4 hours. The incubation and absorption times selected by me are based on these studies.

Biochemical Distribution of Absorbed Glycine

In the in vitro studies the distribution of radioactivity from absorbed Glycine-C^{14} into the 4 main biochemical fractions was approximately the same for both H. diminuta and H. megalocephala. Ninety-two to 97% of this glycine remained in the free amino acid fraction, while 3 to 6% was incorporated into the protein fraction. The lipid fraction contained 1.9% of the absorbed radioactivity, and 0.10% of the radioactivity was incorporated into the glycogen fraction (Table 2). Neither the relative concentration of, or the position of the label in, the glycine, nor the incubation in the presence of bile had a significant effect upon the distribution of the absorbed radioactivity into the 4 biochemical fractions. These results essentially duplicate those reported for H. diminuta by Graff, et al. (1965).

The distribution into the biochemical fractions was somewhat different in the in vivo absorptions. In normal hosts given the glycine by stomach tube, the percentage distribution of the glycine label in H. diminuta was 87 to 90% in the free amino acids, 12% in protein, 0.06% in lipids, and 0.12% in glycogen. In H. megalocephala the distribution was 91 to 92% in the free amino acids, 8% in protein, 0.77% in lipids, and 0.6% in glycogen (Table 4). Greater incorporation of the absorbed C^{14} into the latter 3 fractions occurred in both worms when the glycine

was given intraperitoneally to normal hosts; the distribution in H. diminuta was 70 to 88% in free amino acids, 29% in protein, 0.35% in lipids, and 0.59% in glycogen. The corresponding distribution in H. megalocephala was 82 to 91% in free amino acids, 15% in protein, 0.69% in lipids, and 1.72% in glycogen (Table 4). Even increased incorporation of the label into the latter 3 fractions occurred in cestodes in hosts whose bile ducts were ligated. This was especially true for the protein fraction. The percentage distribution into the respective fractions of H. megalocephala in bile duct ligated hosts given the glycine by stomach tube was 66 to 68% in free amino acids, 31% in protein, 0.56% in lipids, and 5.3% in glycogen. Limited data indicate similar distributions in H. diminuta in the ligated hosts (Table 6).

Clearly the absorbed glycine or labeled carbon therefrom is distributed more greatly among the protein, lipid, and glycogen fractions of the cestodes if the absorption takes place in vivo, and the deprivation of bile apparently stimulates even increased incorporation in these fractions. Slight differences and variations in the results between samples of the Glycine-C^{14} are thought to be due mainly to differences in glycine concentration between these samples. All 3 molecular species of the Glycine-C^{14} were apparently metabolized in the same ways by both cestodes.

Evidence provided by this study strongly indicates that cestodes can satisfy a significant portion of their amino acid requirements from the host bile contribution to the host-parasite interface.

CHAPTER V

SUMMARY

In vitro studies of Glycine-C^{14} absorption by Hymenolepis diminuta and Hymenolepis megalocephala sp. n., both reared in cotton rats (Sigmodon hispidus), revealed that the latter species was slightly more efficient in glycine absorption than the former in aerobic incubations, and that 10%, v/v final conc., host gall bladder bile inhibited the absorption in H. diminuta by 19 to 68%, and in H. megalocephala by 51 to 73%, with the inhibition varying inversely with the concentrations of the labeled glycine used. The distribution of the absorbed Glycine-C^{14}, or radioactivity therefrom, into the 4 major biochemical fractions of the cestodes was similar in both cestodes, at free amino acids 92 - 97%, protein 1.9 - 6.2%, lipid 0.1 - 1.9%, and glycogen 0.008 - 0.1%. Neither the position of the label in the Glycine-C^{14} nor the incubation in the presence of 10% bile had a significant effect on the distribution of the label into the 4 biochemical fractions. Apparently glycine with the label in position 1 or 2, or uniformly labeled, was metabolized by the same pathways.

In vivo studies revealed that both species absorbed 24 to 92% more Glycine-C^{14} when it was given to normal hosts by stomach tube rather than by intraperitoneal injection, but the absorbed radioactivity is distributed more broadly into the protein, lipid, and glycogen fractions

of the cestodes when administered by intraperitoneal injection. Greater incorporation into protein occurred in H. diminuta, but greater incorporation into lipid and glycogen occurred in H. megalocephala. In these studies with normal hosts the intestinal mucosa absorbed considerably more labeled glycine than did the harbored cestodes regardless of the route of administration. The concentration of the Glycine-C^{14} into the bile was greatest in the intraperitoneal administrations.

A few experiments were completed in which Glycine-C^{14} was administered by stomach tube to infected hosts whose common bile duct was ligated 24 hours prior to experimentation. In these experiments the absorption by the host mucosa was much greater than that of the cestodes, and the concentration of Glycine-C^{14} into the bile approached, and in one experiment exceeded, the level absorbed by the cestodes. The greatest incorporation of the absorbed glycine into the protein, lipid, and glycogen fractions of the cestodes occurred in the bile duct ligated host experiments.

The results of this study support the view that cestodes can satisfy a good portion of their amino acid requirement from the host bile.

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DISSERTATION

IN VITRO AND IN VIVO GLYCINE ABSORPTION BY TWO SPECIES OF *HYMENOLEPIS* (CESTOIDEA)

In vitro studies of Glycine-C^{14} absorption by *Hymenolepis diminuta* and *Hymenolepis megalocephala* sp. n., both reared in cotton rats (*Sigmodon hispidus*), revealed that the latter cestode was slightly more efficient in glycine absorption than the former in aerobic incubations. Further, 10%, v/v final concentration, host gall bladder bile inhibited the absorption in *H. diminuta* by 19 to 68%, and in *H. megalocephala* by 51 to 73%, with the inhibition varying inversely with the concentrations of the labeled glycine used. The distribution of the absorbed Glycine-C^{14}, or radioactivity therefrom, into the 4 major biochemical fractions of the cestodes was similar in both cestodes, at free amino acids 92 - 97%, protein 1.9 - 6.2%, lipid 0.1 - 1.9%, and glycogen 0.008 - 0.1%. Neither the position of the label in the Glycine-C^{14} nor the incubation in the presence of 10% bile had a significant effect on the distribution of the label into the 4 biochemical fractions. Apparently glycine with the label in positions 1 or 2, or uniformly labeled, was metabolized by the same pathways.

In vivo studies revealed that both species absorbed 24 to 92% more Glycine-C^{14} when it was given to normal hosts by stomach tube rather than by intraperitoneal injection, but the absorbed radioactivity is more broadly distributed into the protein, lipid, and glycogen fractions of the cestodes when administered by intraperitoneal injection. Greater incorporation of the glycine into protein occurred in *H. diminuta*, but greater incorporation into lipid and glycogen occurred in *H. megalocephala*. In these studies with normal hosts the intestinal mucosa absorbed considerably more labeled glycine than did the harbored cestodes regardless of the route of administration. The concentration of the Glycine-C^{14} into the bile was greatest in the intraperitoneal administrations.

A few experiments were completed in which Glycine-C^{14} was administered by stomach tube to infected hosts whose common bile duct was ligated 24 hours prior to experimentation. In these experiments the absorption by the host mucosa was much greater than that of the cestodes, and the concentration of Glycine-C^{14} into the bile approached, and in one experiment exceeded, the level absorbed by the cestodes. The greatest incorporation of the absorbed glycine into the protein, lipid, and glycogen fractions of the cestodes occurred in the bile duct ligated host experiments.

The results of this study support the view that cestodes can satisfy a good portion of their amino acid requirements from the host bile.