THE CONTRIBUTIONS OF CLARK P. READ ON THE ECOLOGY OF THE VERTEBRATE GUT AND ITS PARASITES

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Clark Read's scientific contributions had great impact on the discipline of parasitology and on biology as a whole. It was Read, more than any other worker, who articulated the concept of the host-parasite interface and defined many of the intricate molecular interactions between symbiotes and their hosts. As Justus Mueller, editor of the *Journal of Parasitology*, recently stated, "Clark Read was Parasitology's ambassador to the fields of physiology, biochemistry and molecular biology."

Some of Read's greatest contributions dealt with physiological interactions between intestinal-parasitic helminths and the vertebrate gut, and it is to these works that the following paragraphs are addressed. In 1950, while a graduate student at the Rice Institute, Clark Read completed a monograph entitled *The Vertebrate Small Intestine as an Environment for Parasitic Helminths,* in which he dealt with the parameters of intestinal physiology of greatest importance to the parasitologist. In this publication Read devoted particular attention to the dynamics of the intestinal environment. He considered contributions made to the gut contents by the stomach, liver, pancreas, and intestinal mucosa, with special reference to the importance of these organs in conditioning the gut lumen as an environment for parasites. He presented strong support for the existence of an exocrino-enteric circulation involving the flow of materials, with the notable exception of carbohydrates, from the blood and from other organs into the lumen of the vertebrate intestine, and subsequent resorption of these materials by the intestine. Read concluded that this flow of organic compounds from the tissues, much of which would be resorbed in areas of the gut distal to the point of secretion, would be available to lumen-dwelling parasites. In addition, Read emphasized the existence of lateral variation in the physico-chemical conditions of the

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intestine, which resulted in a luminal environment with one set of parameters, and a paramucosal environment possessing a different set of physico-chemical conditions. He defined the paramucosal-lumen as "that portion of the lumen of the intestine which is immediately adjacent to the mucosa," and reviewed research that demonstrated differences in the pH, osmotic pressure, oxygen tension, and concentrations of organic and inorganic substances between this area and the center of the intestinal lumen. He also described the paramucosal-lumen as an area of the intestine that may have a close physiological resemblance to the intracellular spaces of the host.

Later, from the work of others and from the results of studies conducted in his laboratory, Read (1970) pointed out that, independent of the quality of food ingested, there is great stability in the molar ratios of free amino acids found in the lumen of the small intestine of the dog, the rat, and the dogfish (Nasset et al., 1955; Nasset and Ju, 1961; Nasset, 1962; Read, Simmons, and Rothman, 1960; Simmons, unpublished). Read (1970) upheld the contention that most important among the sources of endogenous nitrogen contributing to stability of the molar ratios of amino acids in the gut of vertebrates are: 1) digestive enzymes and other secreted proteins found in the saliva, gastric juice, pancreatic juice, intestinal juice, and bile; 2) sloughed off cells of the intestinal epithelium; and 3) the bidirectional flux of free amino acids across the mucosa. In addition, it was noted that fatty acids of the gut were diluted by lipids from exogenous sources, although homeostasis was not as precise as that seen with amino acids (Ginger and Fairbairn, 1966; Kilejian et al., 1968). Maintenance of stable amino acid and fatty acid molar ratios in the intestinal lumen, Read (1970) stated, was an extremely important characteristic of the environment of gut parasites. As was demonstrated by Read, Rothman, and Simmons (1963) for the tapeworm *Hymenolepis diminuta*, competition between different molecular species in mediated absorption (active transport) is a function of molar ratios and not of absolute quantities. Read stated that

> the membrane transport systems of the parasite are exposed to relatively constant molar ratios, and the molar ratios of amino acids absorbed must remain relatively constant in a given host. . . . the parasite, at least in the case of tapeworms, seems to have no mechanisms for establishing or maintaining relative constancy in amino acid ratios relative to the external surface. We are led to the almost inescapable conclusion that parasitism by *Hymenolepis* is not only concerned with the food obtained from the host, but with parasitism of those mechanisms of the host concerned with regulating the molar ratios of amino acids. It is suggested that the basis of this parasitism is considerably more subtle than the obtaining of simple chemical compounds to satisfy nutritional requirements. Unquestionably, nutritional requirements must be and are met, but of similar significance will be dependence on maintenance by the host of a mixture of nutrients compatible with the coupling of absorption and synthetic mechanisms. It may be said that competition between amino acids for transport into cells is clearly of importance for worm metabolism, even though the actual amino acid requirements are not known. Although a given amino acid might not be required by the worm, the presence of this amino acid may regulate, by competition, the rate at which a required amino acid is taken up from the medium.

> Consideration of the relationship of the membrane transport systems of tapeworms to the maintained steady state amino acid ratios of the environment leads to the conclusion that these worms parasitize a physiological control mechanism of the host. Many animal parasites, almost certainly tapeworms, may have certain regulatory capacities lacking or rudimentary. This implies a functional integration with the host which may prove to be of greater consequence than such features as novel, absolute nutritional requirements. It would represent marked integration with regulatory mechanisms of the environment, rather than independence from the environment, for a maintenance of a steady state in the parasite. (1970:190)

Clark Read had long contended (1950:77) that "In order to understand the host-parasite relationships of intestinal helminths we must separately investigate the physiology of the host and of the parasite.... Following such study a resynthesis of host and helminth physiology will reveal entirely new concepts relative to intestinal parasitism." Along these lines Dr. Read devoted much of his scientific energy, from 1950 to the time of his death, to study of the physiology of helminth parasites of the vertebrate gut.

Between 1952 and 1959 Read published several papers dealing with the enzymology, carbohydrate metabolism, and biology of tapeworms. After assuming his first faculty position at U.C.L.A., he used biochemical techniques to demonstrate the presence of cytochrome oxidase and succinic dehydrogenase in the rat tapeworm, *Hymenolepis diminuta*. This was the first report of the former enzyme from a cestode (Read, 1952). An investigation of anaerobic dehydrogenases in the same tapeworm (Read, 1953) demonstrated pyridine-nucleotide-linked enzymes (catalyzing the oxidation of a number of different organic acids) and fumarase and cytochrome-linked α-glycerophosphoric dehydrogenases.

Upon accepting a position at the School of Public Health at Johns Hopkins University, Clark Read began work on the carbohydrate metabolism of helminth parasites of the vertebrate gut. He (1956) showed that in *H. diminuta* aerobic respiration was stimulated or supported by glucose, succinate, malate, glycerophosphate, and malonate. The end-products of anaerobic metabolism in *Hymenolepis* were delineated, and Read showed that this worm carried out glycolysis independent of the glucose concentration in the medium. In reference to the latter observation he stated, "This indicates that the worm is capable of deriving benefit from glucose at very low concentrations." As will be seen, it was through his studies of the "active transport" of nutrients by helminth parasites that Read made some of his greatest contributions to biology.

To determine the function of carbohydrate metabolism in the microecology of helminth parasites of the vertebrate gut, Read began a series of studies entitled "The role of carbohydrates in the biology of cestodes" (Read and Rothman, 1957a, b, and c). The size of the tapeworm *H. diminuta* was affected by the quality of carbohydrate ingested by the host. Significant

differences in the volume of gravid segments were noted in worms from hosts allowed to consume different carbohydrates. Morphological changes induced by specific carbohydrates were accompanied by changes in the reproductive rates of worms. *Hymenolepis* from hosts on diets containing only starch, of all the carbohydrates tested, demonstrated the best size maintenance and rates of reproduction. Less effective in supporting worm growth and reproduction were host diets in which glucose, dextrins-maltose, or sucrose was the sole dietary carbohydrate. Fructose alone depressed worm reproduction to a level equal to that of worms from hosts receiving no carbohydrate at all in their diets. Eggs produced by worms from hosts on a sucrose diet were abnormal in shape and reduced in size. The reproductive rates and growth of worms from hosts receiving suboptimal amounts of dietary starch, plus fructose, were greater than those of worms from hosts on a similar starch diet without fructose. Read (1959) suggested that fructose may interfere with absorption by the host gut of the products of starch hydrolysis. The quality of carbohydrate in the host's diet also affected the growth of *Hymenolepis citelli* and *Hymenolepis nana.* As in the case of *H. diminuta*, sucrose did not support the growth of these worms as well as starch (Read, Schiller, and Phifer, 1958). Reduction in the size and number of *Lacistorhynchus tenuis* occurred in dogfish starved for seven days. When the host was given carbohydrate orally during the starvation period, however, the size and number of this tapeworm were normal (Read, 1957). Read and Phifer (1959) tested the hypothesis that competition for usable carbohydrates by tapeworms is the factor responsible for limiting the size of individual worms in infections of varying intensities. They found that under conditions of crowding, in hosts given a worm-limiting amount of starch or sucrose, the weight of individual *H. diminuta* decreased in the presence of increasing numbers of worms. In this same study animals were infected with one worm of each of two species of tapeworm (*H. diminuta* and *H. citelli*). *Hymenolepis citelli* from hosts on low-starch diets showed an equal reduction in size whether or not *H. diminuta* was present. *H. diminuta*, on the other hand, became smaller in the presence of *H. citelli* than in its absence (Read, 1951). Read and Rothman (1958a) observed a marked reduction in weight of the acanthocephalan *Moniliformis dubius* from rats fed a low-carbohydrate diet. The polysaccharide content of worms was dramatically affected by the quality of carbohydrate included in the host diet. Diurnal fluctuations in the polysaccharide content of *Moniliformis* were correlated with the feeding habits of the host.

Although some six or seven sugars were examined, *Hymenolepis diminuta* (Read, 1956), *H. nana*, *H. citelli*, *Mesocestoides latus* (Read and Rothman, 1958b), *Calliobothrium verticillatum*, and *L. tenuis* (Read, 1957) metabolized only glucose and galactose to a significant extent. On the other hand, *Cittotaenia* sp. (Read and Rothman, 1958b) utilized maltose as well as sucrose.

In the final paper of this series of publications dealing with carbohydrate metabolism in cestodes, Read (1959) presented the following conclusions and hypotheses: 1) carbohydrates in the gut contents of the host are used by tapeworms to fulfill carbohydrate requirements for their growth and reproduction; 2) there are strict limitations in the quality of carbohydrates that tapeworms can utilize to support growth and reproduction; 3) the rate of growth and reproduction and the size attained by worms is dependent on the type of carbohydrate eaten by the host; 4) competition for available carbohydrate in the host gut may be the basis for the crowding effect seen between worms of the same and of different species; 5) of the three carbohydrates tested (glucose, sucrose, and starch), starch was best in supporting the growth and reproduction of tapeworms. Read concluded that since glucose appeared to be rapidly absorbed by the host in the upper part of the small intestine, and since the enzyme sucrase was primarily located in the same area of the vertebrate gut, the quantity of carbohydrate usable by the worm in hosts on diets including only glucose or sucrose was limited. On the other hand, the more complicated series of hydrolytic events involved in the degradation of starch to glucose would allow passage of usable sugar to the area of the gut occupied by tapeworms and would present worms with usable sugar for longer periods of time; 6) the imposition of the dynamics of vertebrate gut physiology on the specific carbohydrate requirements of tapeworms is of importance in cestode distribution, host age, or sex-linked resistance and speciation in tapeworms. The feeding habits of the host are apparently of importance to establishment of a cestode in a particular host as well as to the completion of the tapeworm life cycle; and 7) competition between host and tapeworm for carbohydrates appears to be negligible, since the parasites are located below the region in the host gut in which greatest absorption of carbohydrates occurs.

Additional studies on the influence of host feeding habits on the biology of tapeworms were carried out in Read's laboratory. *Hymenolepis diminuta* underwent a circadian longitudinal migration in the small intestine of the rat, which was related to the feeding of the host (Read and Kilejian, 1969). Further observations on this phenomenon (Chappell, Arai, et al., 1970) revealed the following three migration phases on the basis of worm age: 1) five- to seven-day-old worms migrated from the middle of the small intestine to its anterior end during host fasting; 2) seven- to eight-day-old worms showed no migration; 3) nine- to fourteen-day-old worms migrated to the posterior end of the host small intestine during host fasting and anteriorly during host feeding. A relationship between the migratory behavior of worms, worm growth, and the "crowding effect" was observed. Read postulated that migratory phase 3 and the crowding effect resulted from intraspecific, and perhaps interspecific, competition for nutrients, probably glucose. His hypothesis was supported by the fact that glycogenesis

was minimal in worms older than seven days during host fasting and was greatest during host feeding. In addition, glycogen levels in young worms fluctuated regularly, whereas glycogen levels in older worms changed diurnally. This difference implied that intestinal glucose concentrations are not limiting to growth in *H. diminuta* until the worms reach a certain mass, at which time competition for glucose influences the positioning of worms in the gut. In smaller, younger worms such competition may not exist, and worm positioning is determined on the basis of the best location in relation to glucose concentrations in the gut as dictated by host feeding habits.

Read, Rothman, and Simmons (1963) stated, "The physicochemical relationships between a host and a parasite will involve a region in space and time which may be termed the host-parasite interface. The host-parasite interface will be intimately involved in determining the nature and extent of integration, and thus the outcome of the relationship, since it represents the region of chemical juxtaposition of regulatory mechanisms of both host and parasite. In the latter regard, two distinct aspects of the interface have regulatory significance: the physicochemical characteristics of the surrounding host fluids, together with mechanisms regulating their composition; and the functional characteristics of surfaces or surface membranes of the parasites themselves. The latter includes the bounding membrane and associated organelles in acellular forms." Parasitism is usually defined as a nutritional relationship. The process by which parasites obtain food should therefore be considered of extreme importance in understanding relationships between parasites and their environment. In order for a material to serve as a nutrient it must be available for metabolism. Since tapeworms possess no digestive tract, it was assumed that nutrients entered the body through the outside surface. The absorptive nature of the external surface of tapeworms had been demonstrated in electron microscopic studies (Rothman, 1963; Lumsden, 1965). Lumsden (ibid.) concluded that the microvilli found on the surface of tapeworms were quite similar to those seen on the surfaces of cells with known absorptive functions, such as the epithelial cells of the vertebrate intestine. Furthermore, the tapeworm tegument possessed physiological activity (see review by Read, 1966) found associated with other absorptive surfaces.

After 1959 much of the research carried out in Read's laboratory aimed at understanding animal parasitism with respect to the chemical exchanges between hosts and parasites. As mentioned earlier, Read considered the host-parasite interface to be a highly significant component of these chemical exchanges. An understanding of host-parasite nutritional relationships on the molecular level would require knowledge of the processes involved in the movement of organic compounds across the parasite surface (Read, Rothman, and Simmons, 1963). Most of the work done on these processes in animal parasites was conducted in Clark Read's laboratory, or in the

laboratories of scientists who had studied under him. Read pursued the following objectives in the studies discussed below: 1) a detailed analysis of membrane transport systems in helminth parasites of the vertebrate gut; 2) study and analysis of the interactions of extrinsic host enzymes with the surface of gut parasites; and 3) study and characterization of intrinsic-bound parasite surface enzymes, with special reference to interaction of such enzymes with membrane transport systems.

Read, Rothman, and Simmons defined "active transport" as a

> term applied to processes exhibiting characteristics which are quite different from those of simple diffusion. The most significant aspect of the process is the movement of a substance across a membrane against a chemical concentration difference—i.e., there is a net movement of solute not attributable to its kinetic energy. Such uphill transport implies the participation of forces other than those of diffusion, and at the present time is the only certain criterion of active transport. Usually, however, the process is characterized by stereospecificity involving competitive inhibitions by chemically similar compounds and inhibitions by poisons of energy metabolism. Rather than being a linear function of concentration difference, the rate of movement of a substance being actively transported most frequently follows saturation kinetics. (1963:155)

Read, Simmons, Campbell, and Rothman (1960) demonstrated the presence of a mediated system of entry (active transport) for amino acids in the gut of the dogfish and the tapeworm *Calliobothrium verticillatum*. Definite differences between the amino acid transport systems of the two were shown; Read and Simmons (1962), however, revealed that competitive inhibition of the uptake of a single amino acid by a mixture of amino acids was similar in kinetic characteristics to that seen in inhibition by a single amino acid. Uptake of some amino acids by *H. diminuta* (Kilejian, 1966; Harris and Read, 1968), *C. verticillatum* (Read et al., unpublished), and the dogfish gut (Read, 1967) were inhibited by previous accumulation of sugars which, in the case of the dogfish intestine, were actively absorbed by the mucosa. Similar to a number of other tissues and organs (Crane, 1965), the dogfish intestine (Read, 1967) possessed sodium-dependent amino acid and sugar transport systems. Potassium interfered with the sugar, but not the amino acid, entry system. Read, Rothman, and Simmons (1963) demonstrated active absorption of amino acids by *H. diminuta* and showed conclusively that various amino acids competed with each other in the process of entry. Study of competition between amino acids for entry into worms, and use of several metabolic poisons, led to proposal of four qualitatively different loci for the active absorption of amino acids by *H. diminuta*. Work with worms of different ages and from different hosts led to the conclusion that important phenotypic changes in the active entry systems for amino acids may occur from exposure to environments provided by different hosts, or as a function of differences in the physiology of worms of different ages. On the basis of these studies, the researchers hypothesized (ibid.) that observed

alterations in the active transport systems in *H. diminuta* may be a result of changes in the relative numbers of qualitatively different transport loci for amino acids. Further characterization of the systems for active transport of amino acids in *H. diminuta* was carried out by Harris and Read (1968), Laws and Read (1969), Woodward and Read (1969), and Pappas, Uglem and Read (1974).

Harris and Read (1969) found that *H. diminuta* incorporated amino acids from the surrounding medium into protein. The sharp decrease in the polysaccharide reserves of starved worms, shown by Read (1956), was accompanied by a decrease in the incorporation of amino acids into the protein of *H. diminuta* (Harris and Read, 1969). Glucose stimulated the incorporation of lysine into proteins of starved *H. diminuta* and of valine into proteins of starved *C. verticillatum*, but inhibited incorporation of valine into the proteins of unstarved *C. verticillatum* (Fisher and Read, 1971). Glucose also inhibited incorporation of lysine into proteins of unstarved *H. diminuta* (Harris and Read, 1969). The effects of available carbohydrate on protein synthesis in *H. diminuta* supported the previous finding of Read (1956) that carbohydrate was required for growth and reproduction in that parasite. Harris and Read concluded that *H. diminuta* possesses a "catabolic carbohydrate metabolism driving an anabolic protein and fat metabolism" (1969:654).

Arme and Read (1969) investigated the host-parasite relationship between the rat and *H. diminuta*, concentrating on the distribution of the non-metabolized amino acids in the host and the parasite, and on bidirectional fluxes of these amino acids *in vivo* and *in vitro*. Following injection into rats, the non-metabolizable amino acid cycloleucine quickly reached a "steady-state distribution" in the tissues of the host and of the parasite. The concentration of cycloleucine was lower in worms than in host serum. High rates of exchange between the host and the parasite were comparable to those seen between the organs and body fluids of the rat. The investigators concluded from these findings that tapeworms parasitizing the vertebrate gut fulfill their amino acid requirements from endogenous host sources, and further, that "the rates of flow of cycloleucine into the gut lumen, and the apparent concentration of this amino acid rapidly attained, together form a powerful argument that the worm lives in an extracellular space which allows access to the amino acid pool of the host body." In addition, they reported that: 1) there was a rapid flow of non-protein amino acids (cycloleucine and α-amino-isobutyric acid) into the lumen of the rat intestine, and the concentrations rapidly reached equilibrium with those of the extra-intestinal body fluids, and 2) administration of a single amino acid of sufficient quantity would produce a gross imbalance in the amino acid composition of the intestinal contents resulting in a rapid influx of endogenous free amino acids. On the basis of these findings, and of the results of studies by Simmons,

Read, and Rothman (1960), Hopkins and Callow (1965), and Nasset and Ju (1961), it was postulated that "the flow of endogenous free amino acids, rather than the digestion of endogenous protein, may be of greater importance in regulating the relative quantities of free amino acids in the gut lumen." In addition, the active influx and efflux of cycloleucine, and interactions of other amino acids with the absorption and outflow mechanisms for this amino acid in the gut of the rat host and in *H. diminuta*, were determined and compared. In contrast to findings with *H. diminuta*, lysine and proline inhibited the uptake of cycloleucine and stimulated its efflux from rat gut.

The active absorption of glucose by intestinal helminths was demonstrated for *H. diminuta* (Phifer, 1960) and *C. verticillatum* (Fisher and Read, 1971). Studies on the role of cations in glucose uptake by *H. diminuta* (Read, Stewart, and Pappas, 1974) and *C. verticillatum* (Fisher and Read, 1971) revealed that the active entry system for this sugar in both worms was sodium sensitive. Read (1961) found that the uptake of glucose by *H. diminuta* was competitively inhibited by galactose and some other monosaccharides. These findings suggested that the glucose transport system in *H. diminuta* was similar to that of the vertebrate intestine (reviewed by Crane, 1960). Fisher and Read (1971) found that *C. verticillatum* took up glucose and galactose but did not transport or metabolize mannose or fructose. The gas phase had no effect on the absorption or accumulation of glucose by this tapeworm, and the optimal pH for the transport of glucose (pH 8.9) was the same as that found in the part of the dogfish gut inhabited by *Calliobothrium*.

Arme and Read (1968) presented evidence for an active transport system specific for short chain fatty acids (less than nine carbon atoms in the hydrocarbon chain) in *H. diminuta*, and Chappell, Arme, and Read (1969) showed this tapeworm to possess a mediated system of entry specific for long chain fatty acids (greater than twelve carbons in the hydrocarbon chain). In the latter study, the uptake of ^{14}C-palmitate was markedly stimulated by the presence of a number of long chain fatty acids. Stimulation occurred only when the concentration of the effector molecules (stimulator) was present at the same concentration as palmitate. The researchers observed a second type of stimulation of palmitate transport, by laurate, which stimulated the uptake of palmitate regardless of the concentration of laurate. Laurate stimulation was considered to be a result of the unique ability of this compound to increase the solubility of fatty acids (Fieser and Fieser, 1959).

The transport of purines and pyrimidines into *H. diminuta* was first investigated by MacInnis, Fisher, and Read (1965), who demonstrated mediated processes for the uptake of several of these compounds and showed inhibitory and stimulatory interactions between structurally analogous molecular species. Pappas, Uglem, and Read (1973b) further characterized the purine-pyrimidine transport systems in *Hymenolepis* and proposed a

3-locus model for the transport of purines and pyrimidines based on inhibitory and stimulatory interactions between the various compounds tested. McCracken et al. (1975) have found that the mediated absorption of certain pyrimidine nucleosides by *H. diminuta* is Na^+ dependent.

Evidence that *H. diminuta* requires water-soluble (B) vitamins for normal growth and development was presented by Platzer and Roberts (1969, 1970). Pappas and Read (1972a, b) investigated the mechanisms for entry of thiamine (B_1), riboflavin (B_2), and pyridoxine into *H. diminuta*. These workers demonstrated the existence of both mediated and non-mediated systems for entry of thiamine into this tapeworm. Riboflavin was absorbed by a specific, active process, and the mediated uptake of the vitamin was inhibited by a number of compounds structurally similar to riboflavin. The absorption of pyridoxine occurred by diffusion. Pappas and Read (1972a, b) pointed out some similarities between vitamin absorption by *H. diminuta* and the mammalian gut. *H. diminuta* transports thiamine and riboflavin and obtains pyridoxine by diffusion, whereas the mammalian intestine actively absorbs only thiamine (Matthews, 1967) and takes up pyridoxine by a non-mediated process. Evidence was presented in support of the hypothesis that vitamins are supplied to worms *in vivo* by the exocrino-enteric circulation of the host.

Read (1970) pointed out that the vertebrate mucosa possesses a brush border facing the gut lumen which has been widely considered to be involved primarily in the absorption of nutrients by the host. Recent evidence, however, (Nachlas et al., 1960; Miller and Crane, 1961; Eichholz, 1967; Eichholz and Crane, 1965; Johnson, 1967) has demonstrated that this surface is also endowed with a capacity for digestion of proteins and a wide variety of sugars. In addition, Read mentioned that a charged mucopolysaccharide layer (glycocalyx) on the surface of the mucosa cells (Ito, 1969) was capable of binding proteins (Bell, 1962) and conferred additional digestive capacity on these cells. This mechanism of potentiation of digestive capacity following adsorption of proteins to the surface of mucosa cells had been referred to by Ugelov (1965) as "contact" digestion. Read (1970) emphasized the potential for a close functional relationship between the absorptive and digestive activities present on the surface of the cells of the mucosa, and he mentioned the work of Crane (1967), which showed that a favorable spatial arrangement of absorptive and digestive components of the membrane would result in a "kinetic advantage" for the absorption of the products of sugar hydrolysis. As has been pointed out earlier, the tegument of *H. diminuta* shows functional and morphological similarities to the mucosa cells of the vertebrate gut and the tegument of this worm possesses a brush border composed of functional microvilli (Lumsden, 1966) and an external mucopolysaccharide coat which adsorbs high molecular weight charged substances (Lumsden, 1972), as well as inorganic ions (Lumsden, 1973; Lumsden and Berger, 1974).

Taylor and Thomas (1968) found an enhancement of amylase activity in the presence of living tapeworms. Read (1973) supported the findings of these authors when he reported a marked increase in the activity of pancreatic α-amylase in the presence of *H. diminuta*. He concluded that this tapeworm adsorbed pancreatic α-amylase onto its epithelial surface. This was supported by the following findings: 1) maximum relative increase in amylase activity was attained at low enzyme concentrations; 2) the increase in activity is reversed by washing the worms; and 3) high molecular weight polycations partially block the effect. Read suggested that adsorption of amylase onto the epithelial surface of *Hymenolepis* may stabilize the enzyme in a configuration that favors the catalytic activity of amylase.

Ruff, Uglem, and Read (1973) found no interactions between the acanthocephalan, *M. dubius*, and pancreatic trypsin, α-chymotrypsin, β-chymotrypsin, or lipase. This worm did not appear to possess intrinsic proteases or lipases. Intact *M. dubius*, however, freshly removed from the host, had amylolytic activity. It was determined that this amylase activity was of host origin. Borgström et al. (1957) and Goldberg et al. (1968) showed that, in the presence of intact mammalian mucosa, proteolytic enzymes of pancreatic origin are inactivated. Reichenbach-Klinke and Reichenbach-Klinke (1970) reported the inactivation of trypsin in the presence of the tapeworm *Proteocephalus longicollis*. Pappas and Read (1972c) found that intact *H. diminuta* inactivated trypsin when incubated with this protein. These authors postulated that a trypsin inactivator, possibly associated with the glycocalyx of *Hymenolepis*, was highly labile and was detached from the surface of worms after combining with trypsin, thus exposing fresh inactivators. Pappas and Read (1972d) offered the same mechanism to account for the inactivation of α- and β-chymotrypsin by intact *H. diminuta*. Ruff, Uglem, and Read (1973) reported inactivation of pancreatic lipase in the presence of intact *Hymenolepis diminuta*; but further characterization of this inhibitory activity prompted the authors to postulate that the enzyme underwent a loose, transitory attachment to the worm surface by weak bonding, perhaps involving van der Waal forces, resulting in inhibition of activity due to 1) enzyme stabilization in a catalytically unfavorable configuration, or 2) blockage of the "active" sites on the lipase molecule.

Rothman (1966) and Lumsden et al. (1968) conducted cytochemical studies that demonstrated the presence of phosphatases localized in or on the tegumentary brush border of *H. diminuta*. Arme and Read (1970) used biochemical techniques to indicate the presence of phosphatase activity on the surface of *H. diminuta* involved in the hydrolysis of hexose diphosphates. These authors stated, "It has become increasingly clear that the surface of *Hymenolepis*, and probably other tapeworms, should be regarded as a digestive-absorptive structure." Dike and Read (1971a, b) further character-

ized surface phosphatase activity in *H. diminuta*. They found that the intrinsic tegumentary phosphohydrolase activity occurred at the interface of the worm and the ambient medium. Evidence was provided which indicated that the surface phosphohydrolases of *H. diminuta* are functionally distinct but spatially proximal to the separate system involved in transporting monosaccharides. These data supported the hypothesis that absorption of glucose-6-phosphate occurs in two steps. The first step involves the hydrolysis of glucose-6-phosphate by a surface phosphohydrolase, and the second step is the mediated absorption of the glucose liberated in the first step. Further evidence was provided by the accumulation of glucose in media containing glucose-6-phosphate and an inhibitor of glucose transport. From additional work it was concluded that the hydrolase and the hexose transport systems were located close to one another, between, or at the base of, the microvilli on the surface of the worm.

An intrinsic, membrane-bound ribonuclease (RNase) was demonstrated in *H. diminuta* (Pappas, Uglem, and Read, 1973a). Properties of host pancreatic RNase and *H. diminuta* RNase were compared. Marked differences in pH optimum for enzymatic activity, ion sensitivities, and some kinetic parameters were observed between enzymes from the two sources.

In Read's laboratory, Uglem et al. (1973) observed surface aminopeptidase (APase) activity in the acanthocephalan *M. dubius*. Study of the interactions between the system for the active transport of leucine and the products of leucyl-leucine hydrolysis implied a spatial arrangement between the APase and the leucine transport locus that conferred a kinetic advantage for absorption of the amino acids liberated. Cystocanth larvae of *M. dubius* had no APase activity. Following a thirty-minute exposure of worms to certain surface active agents, however, APase activity was found in larvae.

The scientific endeavors of Clark P. Read, summarized above, provided a large amount of information on the physiology of the vertebrate gut and of its helminth parasites. More importantly, by integrating the prodigious accumulation of data emanating from his and his students' laboratories in these two areas, he significantly advanced our understanding of the molecular basis of changes in the intestinal environment resulting from interactions between hosts and parasites.

Clark Read will be remembered as an outstanding individual and as "the most famous and influential American parasitologist of his age and period" (Simmons, 1974). His short scientific career was marked by the highest level of research productivity.

Present and future generations of investigators in parasitology, intestinal physiology, and symbiosis have suffered an irreparable loss with the passing of Clark P. Read. At the same time, they profit from a legacy that should inspire further advances in these areas.

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