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Chemotherapy of Parasitic Worms: New Biochemical Strategies

Tag E. Mansour

Parasitic helminth infections are widespread throughout the world. It is estimated that 180 million people are infected with the blood flukes *Schistosoma*, 650 million with *Ascaris*, 450 million with *Ancylostoma*, 250 million with *Filaria*, and 20 million with *Onchocerca*, not to mention many other varieties of less prevalent helminth infections (*1*). Most of the victims with these diseases also harbor other parasites: protozoal, bacterial, or viral. At a time when there has been considerable progress in combating major diseases in the developed world, parasitic infections stand as a major obstacle to economic progress and a better life in developing countries. There has been a lack of interest in this problem among scientists in the West and, as a result, the field has not benefited from many of the advances in biology and medicine.

In a previous review of the pharmacology and biochemistry of parasitic helminths (*2*), I emphasized the metabolic differences between these organisms and their hosts. We now understand that the metabolic pathways of these parasites may also vary in different parasite species (*3*). Cohen (*4*) proposed a strategy for the chemotherapy of infectious diseases utilizing these biochemical differences. Inhibition of enzyme systems that are crucial to the parasite but not to the host may be the basis of a rational approach to the chemotherapy of parasites. Paul Ehrlich (*5*) laid the foundation for such an approach.

During the last two decades, investigations in the field of cellular regulatory biology have enhanced our knowledge of many basic principles in enzyme and hormone control in different organisms. Progress in understanding control mechanisms in parasites has been scanty, however, partly because of the difficulty of culturing these organisms in well-defined media.

In this article, I focus my discussion on the motility of parasitic helminths and the regulation of metabolism. I also include a brief discussion on chemotaxis, a process that is poorly understood in parasites but is nevertheless an important regulatory mechanism that deserves more attention. Examples of the action of certain drugs at the level of regulatory sites are given to illustrate the possible utilization of these sites for the selection of new antiparasitic agents.

Regulation of Motility in Parasitic Helminths

The survival of most parasitic helminths in their natural habitat is largely dependent on their ability to remain in situ when exposed to peristaltic movement in the case of intestinal parasites, or the movement of blood or lymph in the case of some systemic parasites. Some parasites have specialized sucker-like organs to move within and attach themselves to the host. When kept in vitro, these organisms show fast and well-coordinated rhythmical movements. These movements help the parasites to stay in their specific host sites and also influence the movement of the food they ingest through their intestinal caeca as

The author is Donald E. Baxter Professor of Pharmacology and chairman of the Department, Stanford University School of Medicine, Stanford, California 94305. Some of the material in this article was presented at a conference on "Pharmaceuticals for Developing Countries" sponsored by the National Academy of Sciences, Institute of Medicine, on 29 to 31 January 1979.

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well as movement of the excretory system contents. Studies on the pharmacology and biochemistry of parasites that have nervous systems have been handicapped by the lack of information on the neuroanatomy and neurophysiology of these organisms. However, the importance of motility to these organisms in maintaining a successful parasitic life has provided an impetus for pharmacologists to use it as a biological indicator of the effectiveness of new chemotherapeutic agents. In some instances, investigators use the same approach for studying parasitic helminth motility as is used in studies of isolated organ systems of vertebrates. Kymographic recordings of motility of *Ascaris* (*6*) as well as the liver fluke *Fasciola hepatica* (*7*) were used for studying the effect of different chemical agents on motility. Recently, more elaborate systems for monitoring motility have been devised in which instruments with multiphotocells are used to measure quantitatively the movement of small parasites such as schistosomes (*8*).

In multicellular organisms it is assumed that certain chemicals and neuromuscular regulators play a key role in the control of communication among nerve cells and from nerves to muscle cells. Two neurotransmitters that have been extensively investigated in parasites are acetylcholine and serotonin (5-hydroxytryptamine). The presence of acetylcholine, cholineacetyltransferase, and acetylcholinesterase were reported in *Fasciola* (*9*), *Schistosoma* (*10*), and subsequently in many other parasites (*3*). Fripp (*11*), using histochemical techniques, identified both enzymes in the central nervous system of male and female schistosomes.

Many pharmacological experiments were done on neuromuscular preparations from large parasites. Baldwin and Moyle (*12*) showed that neuromuscular preparations from *Ascaris* contracted in the presence of acetylcholine. Nicotine was more potent than acetylcholine in reproducing this cholinergic effect. In preparations from *Fasciola*, the cholinesters carbachol, acetylcholine, and methylcholine, in addition to physostigmine and prostigmine, relaxed the preparations so that the end result was either complete paralysis or a marked reduction in the amplitude of contraction (*7*, *9*). Chemical agents that inhibit acetylcholinesterase, such as physostigmine, sensitized the preparation to the action of acetylcholine. Such results indicate the presence of cholinergic receptors in these parasites. Acetylcholine receptors in trematodes may be different from those found in mammalian synapses containing nicotinic or muscarinic receptors. Neither atropine nor *d*-tubocurarine, two of the most active cholinergic blocking agents on mammalian synapses, affected the neuromuscular activity of these parasites. Thus the regulatory processes at the cholinergic receptors in the parasites appear to be different from those in the host. Even among parasitic helminths as a whole, differences between species with respect to response to neuromuscular drugs is well demonstrated when one compares the pharmacological results obtained from a nematode preparation with those obtained from a trematode (*2*).

Pharmacological evidence for the presence of serotonin receptors in trematodes first came from experiments with *F. hepatica*. Motility of the liver fluke was stimulated by the application of serotonin, lysergic acid diethylamide (LSD), and related indoleamines (*13*, *14*). The effect was peripheral and not mediated through the parasite's central ganglion. Bromolysergic acid diethylamide, an analog of LSD that has a bromine atom in the second position, depressed the rhythmical movement of the fluke and antagonized the stimulant action of serotonin and of LSD. Similar effects were found in other parasitic flatworms. Serotonin stimulated the rhythmical movement of *Schistosoma mansoni*, *Chlonorchis sinensis*, and *Taenia pisiformis* (*2*). Two different groups of investigators (*15*, *16*) verified these findings with *S. mansoni*, and similar results were obtained with the cestode *Mesocestoides corti* (*17*). Hillman *et al.* (*18*) investigated the effects of two compounds that are closely related to LSD and serotonin, methysergide and dihydroergotamine, on the motility and metabolism of *S. mansoni*. Both agents stimulate the motility of these parasites at high concentrations and have a depressant effect at lower concentrations. Presumably, these effects are mediated through the serotonin receptors and are identical to those previously reported with the related trematode *F. hepatica* (*13*, *14*).

Serotonin is an indoleamine that is ubiquitous among invertebrate tissues. Erspamer (*19*) and Welsh and Moorhead (*20*) demonstrated the presence of serotonin in several invertebrate nervous systems. In our laboratory, using a bioassay (*2*, *21*) and fluorometric methods (*22*), we reported that the indoleamine is present in *F. hepatica* (*2*, *21–23*). Intact liver flukes were capable of synthesizing serotonin from 5-hydroxytryptophan but not from tryptophan (*21*, *22*). Although 5-hydroxytryptophan decarboxylase and

Summary. Many chemotherapeutic agents that are effective against parasitic helminths affect cellular regulatory sites that control motility, metabolism, chemotaxis, and egg formation. Serotonin receptors are present in several species of parasitic flatworms and appear to participate in the regulation of motility and carbohydrate metabolism. In *Fasciola hepatica* these receptors are coupled to an adenylate cyclase through a cellular component that requires guanosine triphosphate. Serotonin is the most potent indoleamine agonist, while lysergic acid diethylamide and its 2-bromo derivative are the most potent antagonists. These studies are revealing additional sites in trematodes that may be important for the development of new and more selective chemotherapeutic agents.

Table 1. The role of serotonin in the regulation of motility and metabolism in parasitic flatworms.

Regulatory process	Parasite	Serotonin action	References
Movement	*F. hepatica*	Stimulant	(*13*, *14*)
Movement	*S. mansoni*	Stimulant	(*2*, *15*, *16*)
Movement	*C. sinensis*	Stimulant	(*2*)
Movement	*T. pisiformis*	Stimulant	(*2*)
Movement	*M. corti*	Stimulant	(*17*)
Carbohydrate metabolism			
Glycogenolysis	*F. hepatica*	Increase	(*40*)
Glycolysis	*F. hepatica*	Increase	(*40*, *41*)
Glycolysis	*S. mansoni*	Increase	(*18*)
Glycogen phosphorylase	*F. hepatica*	Activate	(*36*)
Protein kinase	*F. hepatica*	Activate	(*49*)
Adenylate cyclase	*F. hepatica*	Activate	(*36*)
Adenylate cyclase	*S. mansoni*	Activate	(*37*)
Phosphofructokinase	*F. hepatica*	Activate	(*51–53*)

Table 2. Chemotherapeutic agents that affect processes regulating parasite motility.

Agent	Parasite	Effects	References
Santonin	*Ascaris*	Neuromuscular paralysis	(*6*)
Piperazine	*Ascaris*	Paralysis; inhibits synaptic transmission by hyperpolarization of membranes	(*27, 28*)
Pyrantel	*Ascaris*	Spastic paralysis; neuromuscular blocking action by depolarization	(*28*)
Methyridin	*Ascaris*	Paralysis; depolarization at the neuromuscular receptors	(*84*)
Tetrachloroethylene	*Ancylostoma*	Neuromuscular excitation	(*85*)
Metrifonate	*Schistosomes*	Paralysis, anticholinesterase	(*32*)
Hycanthone	*Schistosomes*	Acetylcholine-like action	(*86*)
Arecoline	*Cestodes*	Paralysis of muscle movement	(*87*)

its activation by pyridoxal phosphate were demonstrated in cell-free extracts of the flukes (*2, 21*), the first enzyme for the synthesis of 5-hydroxytryptophan from tryptophan has not been identified in the liver fluke. This is not surprising since the organism is known to be an anaerobe (*24*). The question of how trematodes synthesize serotonin from the essential amino acid tryptophan will have to be resolved. It is possible that 5-hydroxytryptophan, or serotonin itself, may be provided to the parasite by the host. A good source of these indoles would be the blood platelets in the hepatic vein from which the flukes obtain blood. Schistosomes have been reported (*25*) to have an uptake mechanism for serotonin. These data (see Table 1) strongly implicate serotonin as a putative neurotransmitter in the regulation of neuromuscular activity in parasitic flatworms.

Chemotherapeutic Agents That Affect Parasite Motility

Several chemotherapeutic agents that are effective against helminths affect specific mechanisms that regulate motility (Table 2). The mode of action of piperazine on *Ascaris* illustrates the idea that within the regulatory mechanisms of neuromuscular function may lie sites of action that could be utilized in selecting effective chemotherapeutic agents. Standen (*26*) demonstrated that *Ascaris* incubated with piperazine is reversibly paralyzed. When the drug was introduced as an anthelmintic it was realized that worms expelled from patients were paralyzed, but the muscular activity of the worms was recovered when they were subsequently incubated in a piperazine-free medium. Norton and DeBeer (*27*) showed that piperazine blocked the effect of acetylcholine on neuromuscular preparations. Subsequently, del Castillo and others (*28*) reported that piperazine increases the resting potential of *Ascaris* muscle and suppresses its pacemaker activity so that flaccid paralysis occurs. Piperazine may be a pharmacological analog of a natural inhibitory transmitter. The molecular identification of such a natural transmitter is a problem that deserves further investigation.

Other evidence that anthelmintic drugs act on cholinergic receptors in nematodes comes from studies of organophosphorus compounds that are potent anticholinesterases and have anthelmintic effects. The mechanisms of action of these compounds have yet to be discovered. The organophosphorus cholinesterase inhibitor Dipterex was used as a pesticide for some years before it was introduced in pure form as a schistosomicide under the name metrifonate (*O,O*-dimethyl-2,2,2,trichloro-1-hydroxyethylphosphanate). Metrifonate was shown to be effective in the treatment of human urinary schistosomiasis caused by *Schistosoma hematobium* but lacked activity in animals and human subjects infected with *S. mansoni* (*29, 30*). The organophosphorus derivative appears to inhibit cholinesterase and acetylcholinesterase in both parasites (*31*). According to Denham and Holdsworth (*32*), metrifonate has a reversible paralyzing effect on both *S. hematobium* and *S. mansoni*. Paralyzing *S. hematobium* causes the flukes to relax their hold in the bladder veins; eventually the parasites are carried via the bloodstream to the lungs. In the case of *S. mansoni*, which are located in the mesenteric veins, the parasites are carried to the liver in the known "hepatic shift." As the effect of the drug wears off, the *S. mansoni* can relocate in the mesenteric veins, whereas relocation for *S. hematobium* from the lung to the urinary bladder veins may be impossible. This is a remarkable example of how social behavior of the parasite could effect the therapeutic effect of chemical agents.

Serotonin and Its Role in Regulation of Metabolism in Flukes

The evidence available thus far indicates that regulation of a metabolic pathway may occur at the hormonal level and through allosteric enzyme control. Because of the symbiotic relationship between host and parasite it has been suggested that hormonal control of a parasite may be geared to the hormones of its host. Indirect support for this idea comes from reports on the effects of thyroxine (*33*) and progesterone (*34*) on the number and size of parasites during establishment of an infection. We have evidence, however, that control of metabolism in parasites is mediated through hormones that are different from those used by the host. The involvement of adenosine-3′,5′-monophosphate (cyclic AMP) as a second messenger has been implicated in some parasites (*35-39*). Superimposed on these two levels of biological control is the control of several allosteric regulatory enzymes that are crucial for cellular metabolism. Accordingly, enzymes act not only as catalysts but also as regulators of metabolic pathways.

The liver fluke *F. hepatica* is being used in our laboratory as a model to study the regulation of carbohydrate metabolism in trematodes. *Fasciola* is an anaerobic organism that metabolizes glucose at a high rate and converts it to volatile fatty acids and CO_2 with only small amounts of lactic acid being formed (*24*). Incubation of the organism with serotonin causes a marked increase in lactic acid production (*40*). The effect of serotonin on glycolysis is independent of its action on motility. Serotonin can increase glycolysis in cell-free extracts (*41*). In addition to its stimulatory effect on glycolysis, serotonin increases glycogen breakdown when glucose is omitted from the fluke medium, and activates glycogen phosphorylase and adenylate cyclase (*36, 40*). In contrast, none of these effects can be mediated by epinephrine or norepinephrine.

The effect of serotonin on glycolysis does not seem to be restricted to *Fasciola*. The indoleamine, as well as its agonists methysergide and dihydroergotamine, increases lactic acid production by schistosomes (*18*). Furthermore, Higashi *et al.* (*37*) reported that adenylate cyclase from schistosomes is activated by serotonin. Thus, in both liver flukes and schistosomes serotonin has an effect on carbohydrate metabolism and on adenylate cyclase similar to that of epinephrine in some mammalian tissues; this is a good illustration of a difference

between the parasite and the host that can be utilized for planning a chemotherapeutic agent. A summary of these effects of serotonin is shown in Table 1.

The Regulation of Adenylate Cyclase by Serotonin in the Liver Fluke

In view of the central role of cyclic AMP in cellular regulation and communication, the enzymes controlling its concentration in the cell deserve thorough study. Such study may reveal new sites in the parasite that are amenable to pharmacological manipulation as well as contribute to our understanding of this regulatory mechanism. The liver fluke probably has the most active adenylate cyclase in nature, and its activity seems to be related to increased carbohydrate metabolism in the organism and to increased motility. The fluke, therefore, may be an ideal model system in which to study the mechanism of action of a hormone such as serotonin that may have a double role: as a putative neurotransmitter and as a metabolic regulator.

Abrahams *et al.* (*42*) in my laboratory showed that the activation of adenylate cyclase by the addition of serotonin to the incubation medium causes a marked increase in the concentration of cyclic AMP in the intact organism (Fig. 1). In the isolated anterior, "head" region of the organism the accumulation of the cyclic nucleotide and the accompanying increase in motility are even more marked and are dependent on the serotonin concentration. Serotonin appears to be the most effective indoleamine in increasing the concentration of endogenous cyclic AMP.

In a study of the molecular mechanisms of adenylate cyclase activation by serotonin, we are attempting to characterize the main components in the system. In nature, adenylate cyclase is present either in a simple form, as in bacteria, or as a complex of several components, as in mammals. The bacterial enzyme, which has been purified to homogeneity (*43*), requires only a catalytic component for its activity. The mammalian enzyme seems to be more complex since there are several molecular entities involved. Studies of mammalian cell mutants (*44*) indicate that the enzyme system has at least three components: hormonal receptors, a guanosine triphosphate (GTP) component, and the catalytic component.

Adenylate cyclase in the liver fluke (*36*, *45*, *46*) is associated with cell membranes and has multiple components for its control (Fig. 2). The basal activity

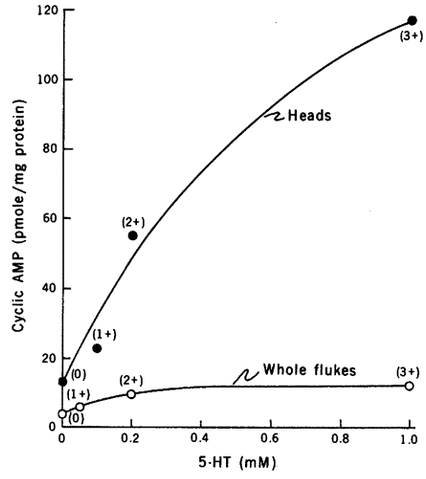

Fig. 1. Endogenous cyclic AMP concentrations in heads of and whole liver flukes, *Fasciola hepatica*, that have been incubated for 5 minutes with various concentrations of serotonin. Estimates of relative motility are shown in parentheses: 0 indicates a resting state and 3+ indicates maximum motility. [Data from Abrahams *et al.* (*42*), by courtesy of *Molecular Pharmacology*]

(without activators) of the enzyme is low, and its activation by serotonin (*45*) is dependent on the presence of GTP (*46*). Guanosine triphosphate is also required for hormonal activation of adenylate cyclase from mammalian sources. The specificity of serotonin activation in flukes was recently confirmed in studies of the relation between structure and activity with different analogs (Fig. 3) (*45*). Serotonin appears to be the most effective indoleamine in activating adenylate cyclase.

Although LSD and its derivatives are poor agonists compared to the indoleamines, they show greater affinity to the serotonin sites (*45*). The most potent derivative was D-LSD, which increased the activity of adenylate cyclase by only 25 percent compared to serotonin. At a concentration of 46 n*M*, D-LSD caused half-maximum activation. Any substitution other than the diethyl group on the amide nitrogen decreased the potency of the derivative. Figure 4 shows that only the D isomer of LSD is effective in activating adenylate cyclase. It is of interest that all of the hallucinogenic substances tested proved to be partial agonists of the receptor. Thus, there may be some similarity between the receptors in the flukes and those in man.

Although many of the indoleamines and the derivatives of LSD acted as antagonists of serotonin, D-LSD and its 2-bromo derivative (BOL) are of special interest. Both D- and L-LSD act as antagonists of serotonin activation, with L-LSD having a very low affinity for serotonin receptors (Fig. 5). Thus both activation of adenylate cyclase and antagonism of serotonin activation are stereospecific processes. The hallucinogenic effect of LSD also shows stereospecificity in that only the D isomer is effective. BOL, which is not an agonist of adenylate cyclase activation, competes for the serotonin activation and inhibits basal (non-activated) enzyme activity. BOL was the most potent antagonist with an inhibition constant, K_i of 28 n*M*. The antagonism was competitive with respect to serotonin. Our kinetic studies

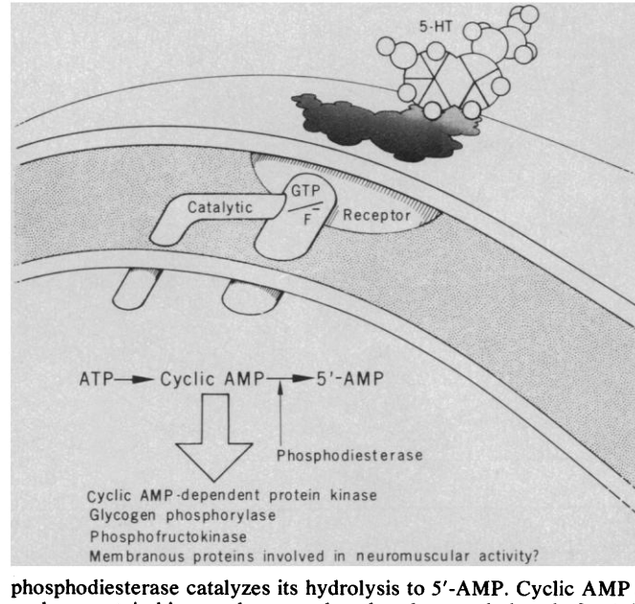

Fig. 2. Role of cyclic AMP as a second messenger. The proposed physiological effects of serotonin (*5-HT*) on trematodes is initiated through binding of the indoleamine to the receptor that is part of a membranous adenylate cyclase. Such a binding activates the catalytic component of the enzyme through the participation of an enzyme component that binds GTP. Fluoride appears to activate the GTP enzyme component. The enzyme catalyzes the synthesis of cyclic AMP from ATP, while phosphodiesterase catalyzes its hydrolysis to 5'-AMP. Cyclic AMP activates enzyme systems such as protein kinase, glycogen phosphorylase, and phosphofructokinase and increases phosphorylation of membranous proteins that may be involved in regulation of neuromuscular activity.

with agonists as well as with antagonists of these sites indicate that these agents act on a single class of sites in the adenylate cyclase system.

The requirement of GTP for serotonin activation prompted us to study the nature of such activation (*46*). First, we found that the guanine nucleotide or one of its analogs is needed for activation not only by serotonin but also by D-LSD (Fig. 6). Adenylate cyclase is highly specific for the guanine nucleotide. Poorly hydrolyzed synthetic analogs of GTP, guanylyl imidophosphate (GppNHp), guanylyl methylenephosphate (Gpp-

Table 3. Activation of protein kinase in fluke heads by serotonin. Protein kinase activity was determined in fluke heads that had been incubated in the presence or absence of serotonin for 5 minutes. [Data from Gentleman *et al.* (*49*), by courtesy of *Molecular Pharmacology*]

Addition to incubation solution	Number of experiments	Particulate fraction			Supernatant fraction		
		Protein kinase activity		Degree of activation	Protein kinase activity		Degree of activation
		Without cyclic AMP	With cyclic AMP		Without cyclic AMP	With cyclic AMP	
None	11	3.5 ± 1.0	11.8 ± 3.6	4.3 ± 0.9	3.2 ± 0.8	14.2 ± 2.2	5.5 ± 0.7
1 m*M* serotonin	10	12.3 ± 3.9*	19.0 ± 3.2*	1.6 ± 0.1*	4.5 ± 1.0	10.2 ± 1.2*	2.5 ± 0.5*

*Significantly different from means of untreated fluke heads ($P < .01$ by the paired-value t-test, one-tailed).

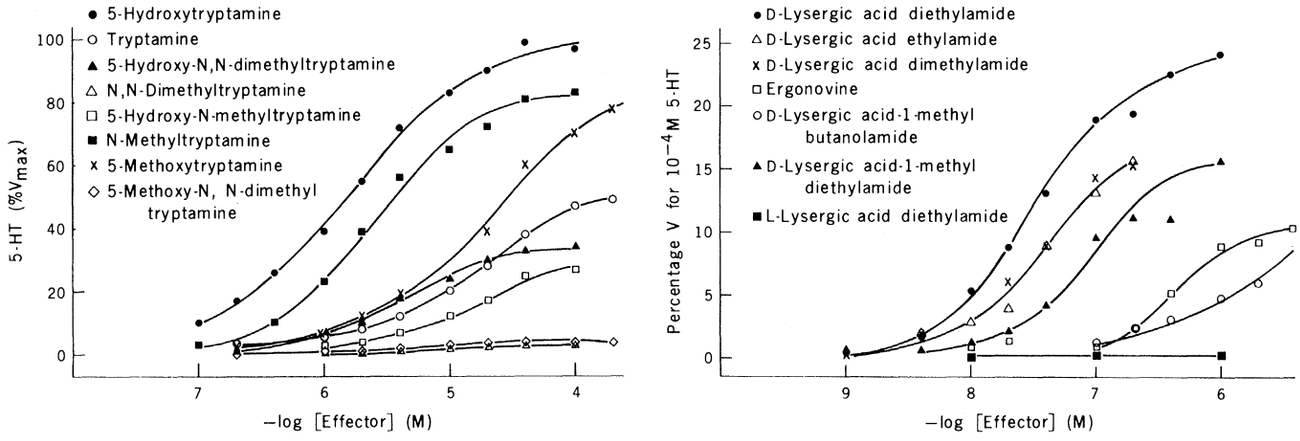

Fig. 3 (left). Relative effect of serotonin analogs in activating adenylate cyclase in the liver fluke. Velocity was normalized as the fraction of maximum velocity (V_{max}) for serotonin (*5-HT*). Abscissa shows concentrations of different effectors. [Data from Northup and Mansour (*45*), by courtesy of *Molecular Pharmacology*] Fig. 4 (right). Relative effect of lysergic acid derivatives. Activity was normalized as that fraction (V) attributable to 100 μM serotonin (*5-HT*). [Data from Northup and Mansour (*45*), by courtesy of *Molecular Pharmacology*]

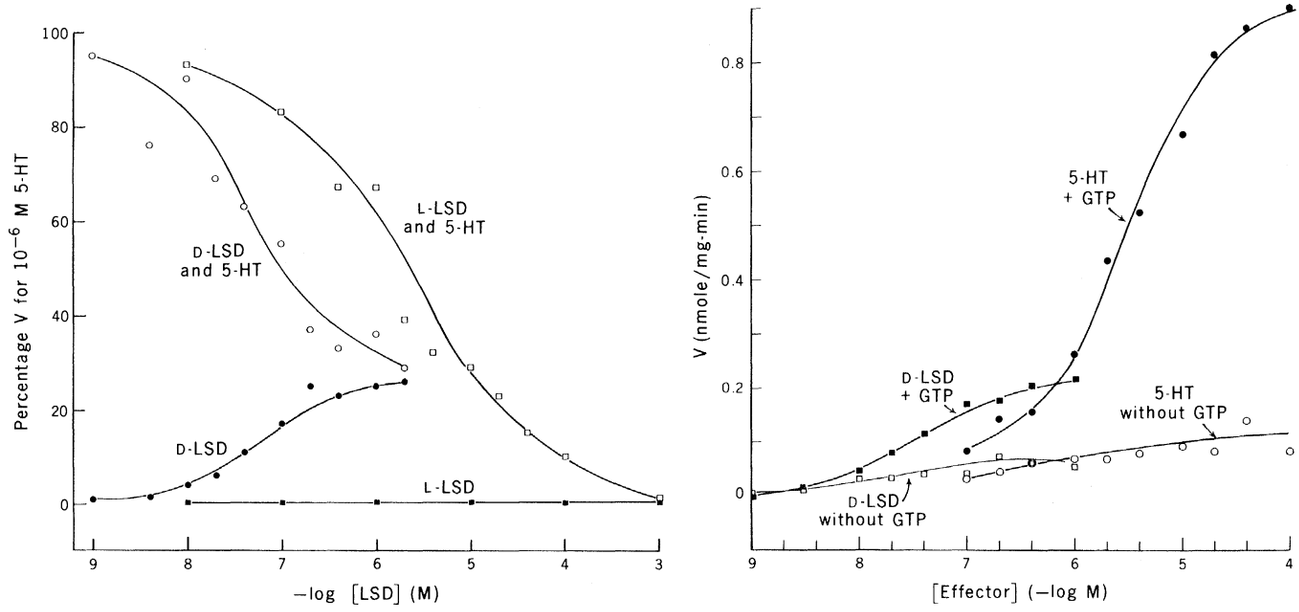

Fig. 5 (left). Antagonism of serotonin activation of D- and L-LSD. Activities have been normalized as the fraction due to 1 μM serotonin (*5-HT*). [Data from Northup and Mansour (*45*), by courtesy of *Molecular Pharmacology*] Fig. 6 (right). Effect of 100 μm GTP on activation by serotonin and D-LSD. The abscissa shows concentrations of serotonin or LSD. [Data from Northup and Mansour (*46*), by courtesy of *Molecular Pharmacology*]

CHp), Cr^{3+}-GTP, and guanosine-5′-(3-*O*-thio)triphosphate (GTPγS) all activated fluke adenylate cyclase in the absence of serotonin. The dependence of adenylate cyclase activation on a guanine nucleotide has been demonstrated in widely disparate cell types and different hormones from vertebrate species.

Cyclic adenosine monophosphate phosphodiesterase is the only enzyme known to hydrolyze cyclic AMP to adenosine 5′-monophosphate (5′-AMP). Both adenylate cyclase and phosphodiesterase regulate the concentration of cyclic AMP in the cell. Phosphodiesterase was found in fluke homogenates (*47*) and appears to participate in the regulation of cyclic AMP in this organism. It was of interest to see whether the effect of conventional phosphodiesterase inhibitors on fluke motility would be similar to that of compounds that activate adenylate cyclase. Indeed, we found that many of the phosphodiesterase inhibitors increase motility in the same way as serotonin and its analogs (*47*). The molecular basis for this effect does not appear to be simple inhibition of the phosphodiesterase, which would increase the concentration of cyclic AMP. Only one of these inhibitors, isobutyl methylxanthine, increased the concentrations of cyclic AMP in flukes. Indeed, many of these agents antagonized the serotonin-mediated increase in endogenous cyclic AMP. In a subsequent investigation we found that many of these agents can inhibit adenylate cyclase itself as well as phosphodiesterase (*48*). Stimulation of motility in these organisms is not simply due to a direct effect on phosphodiesterase. Although the specific mechanism by which cyclic AMP affects fluke motility has not been elucidated, phosphodiesterase appears to offer a site that can be affected with chemical agents that may influence motility of the parasite.

Activation of Protein Kinase by Serotonin in Flukes

Some of the effects of hormones are known to be mediated by cyclic AMP through activation of protein kinases that phosphorylate specific proteins (Fig. 2). Epinephrine activation of glycogen phosphorylase provides an example of such a control mechanism. Studies in our laboratory by Gentleman *et al.* (*49*) demonstrated the presence of a protein kinase both in the particulate and the supernatant fractions of *F. hepatica*. The enzyme activity was two to five times greater in the anterior (head) end of the fluke than in the posterior end. The fluke protein kinase was highly sensitive to cyclic AMP: half-maximum activation of the enzyme in both cellular fractions occurred with 0.1 to 0.4 μM concentrations of cyclic AMP, concentrations that can be found in the fluke. Incubation of the flukes with serotonin activated the protein kinase in both the particulate and the supernatant fractions (Table 3). The results may indicate that, as in mammalian cells, some of the physiological effects of serotonin are mediated by cyclic AMP activation of protein kinase. Such an effect may also apply to other trematodes, since *S. mansoni* was reported to contain this enzyme as well as an adenylate cyclase that is activated by serotonin (*37*). Substrates for the protein kinase have not yet been isolated and identified.

Control Mechanisms in Regulation of Phosphofructokinase

In every metabolic pathway there are rate-limiting reactions that control the flux of the substrates. The enzymes catalyzing these reactions are usually allosteric proteins that exhibit complex kinetics that are complementary to the metabolic needs of the cell. One enzyme that has drawn our attention is phosphofructokinase. This enzyme, which catalyzes the physiologically irreversible phosphorylation of fructose 6-phosphate to fructose-1,6-diphosphate with adenosine triphosphate (ATP) as the phosphate donor, regulates the glycolytic flux in higher organisms [see (*50*)]. Evidence suggests that serotonin activates phosphofructokinase in the liver fluke in addition to activating adenylate cyclase (*41, 51*). Incubation of intact flukes with serotonin decreases the concentration of fructose 6-phosphate and increases the concentration of fructose-1,6-diphosphate. Furthermore, the specific activity of phosphofructokinase increases after incubation with serotonin. The effect of serotonin on phosphofructokinase may thus be mediated through cyclic AMP, and we have shown that this activation can occur in two different ways (*51–53*). First, we found that the cyclic nucleotide activated a concentrated inactive preparation of phosphofructokinase. Under these conditions a direct effect of cyclic AMP on the enzyme during assay was excluded because the final dilution in the assay mixture was below effective concentrations of the nucleotide. Activation of the enzyme under these conditions appears to involve a conversion from a low molecular form ($S_{20,w} = 5.5$) to a high molecular form ($S_{20,w} = 12.8$). More information concerning the nature of the fluke enzyme will have to await its purification.

Once activated, the phosphofructokinase of the fluke is subject to all the allosteric kinetics that are relevant to mammalian phosphofructokinase. This includes inhibition by one of its substrates, ATP. The ATP-inhibited enzyme can be "de-inhibited" by cyclic AMP and fructose-6-phosphate (*51, 53*). Thus the parasite enzyme is well endowed with all the control mechanisms that are necessary for reversible conversion from active to inactive form through changes in both the tertiary and quaternary structure of the protein, a classical example of a regulatory enzyme.

Although the ATP-dependent phosphofructokinase is present in almost all the parasites that have been investigated, *Entamoeba histolytica* contains the enzyme in only trace amounts. Instead, these parasitic protozoa have a phosphofructokinase that utilizes pyrophosphate (PP_i); this newly discovered enzyme catalyzes the reaction fructose 6-phosphate + $PP_i \leftrightarrows$ fructose 1,6-diphosphate + P_i. Its activity in amoebal extracts is more than tenfold greater than the flux through the glycolytic pathway (*54, 55*). The allosteric kinetics of this enzyme have not been studied. This is a striking example of how an organism can salvage the large reserves of pyrophosphate efficiently to synthesize energy-rich ATP through the glycolytic scheme. It also illustrates how an identical reaction can be metabolized by two completely different enzymes that meet the metabolic needs of each organism.

Selectivity of Antimonial Inhibition to Phosphofructokinase

Because of its critical role in the regulation of glycolysis, phosphofructokinase has been implicated as the site of action of an important group of antischistomal agents, the antimonials. Although oxygen seems to be used by schistosomes for oxidative phosphorylation (*56*), experiments in vitro indicate that the survival of schistosomes is dependent on energy from glycolysis (*57*). Glycolysis in intact worms is inhibited by the trivalent antimonials stibophen and antimony potassium tartrate (tarter emetic). The phosphofructokinase of parasites is comparatively more sensitive to inhibition by these antimonials than is the mammalian phosphofructokinase (*58, 59*). Similar selective inhibitory effect of the antimonials on the adult filariids *Dipetalonema witei* (= *viteae*) and *Brugia pahangi* was recently reported

(*60*). These parasites are metabolically similar to the schistosomes in that they are homolactate fermenters. Helminths such as *Ascaris* and *Hymenolepis diminuta*, which are not homolactate fermenters, also exhibit phosphofructokinase activities that are susceptible to inhibition by the trivalent antimonial stibophen. This is in direct contrast to the phosphofructokinases from mammalian tissues that are considerably more resistant to inhibition by the antimonials. All these findings suggest a broad spectrum of activity of the antimonials against parasitic helminth phosphofructokinases. The selective inhibitory effect of these agents appears to extend to phosphofructokinase from the protozoal parasite *Trypanosoma rhodesiense*, which was also reported to be exceptionally sensitive to inhibition by stibophen (*61*). Dissimilarities in the sensitivity of parasite phosphofructokinases on the one hand and the host enzyme on the other suggest structural differences. These structural differences are obviously important and should be characterized.

Other Regulatory Sites Affected by Anthelmintic Agents

Schistosomes seem to depend primarily, if not exclusively, on the metabolism of preformed purines to satisfy their need for purine nucleotides (*62*). Utilizing this property, Jaffe *et al.* (*63*) found that the purine analog tubercidin (7-deazoadenosine) had a potent antischistosomal effect. Tubercidin caused early separation of paired adults and alterations in their muscular activity pattern, and inhibited their egg-laying ability. When administered intraperitoneally to *S. mansoni*-infested mice, tubercidin caused 20 to 30 percent mortality among the parasites. The mechanism of action of tubercidin has been ascribed to inhibition of the utilization of adenosine for adenine nucleotide formation (*64*).

Some parasites, such as schistosomes, deposit eggs with harmful spines within the veins of their hosts. Elimination of these eggs causes much damage and bleeding. Studies on the biochemistry of egg development could be of importance in identifying drugs that interfere with parasite egg-laying capacity. In their studies on the effect of chemical agents on catecholamines in the parasite *S. mansoni*, Bennet and Gianutsos (*65*) reported that disulfuran, when given to schistosome-infected mice, increases the concentrations of dopamine and decreases norepinephrine to almost nondetectable concentrations in schistosomes. While no noticeable change in motility of the parasites was observed, such treatment resulted in abnormal egg production. This effect appears to be related to inhibition of the enzyme phenol oxidase, which was first shown to be present in *F. hepatica* (*66*) and was subsequently found in schistosomes (*65*). Phenol oxidase has a variety of substrates and may be involved in the oxidation of an as yet undetermined catechol substrate that is necessary for egg formation. The effect of disulfuran on egg formation by the parasites may be due to inhibition of the activity of this enzyme. Studies on the biochemistry of egg formation in the parasite may elucidate other sites that are amenable to selective inhibition by chemical agents.

Chemotaxis: Models in Prokaryotes and Eukaryotes

Chemotaxis is a sensory process that enables an organism to respond to favorable and noxious agents in the environment. The process is being extensively investigated in prokaryotes (*67*, *68*), and it appears to involve a receptor, a sensing mechanism that is temporal in nature, and movement of the organism in response to the stimulus. Adler and his co-workers (*67*) showed that *Escherichia coli* exhibit chemotactic responses to specific hydrophilic amino acids and to specific sugars. These responses are independent both of the metabolic pathway and of the permeases involved in the transport of these sugars. Studies on eukaryotic cells are less advanced than those on bacteria. Chemotactic responses of leukocytes to various complement-derived factors and by-products of the inflammatory response, and to *n*-formyl methionine-containing peptides, have been reported (*69*). In addition, the process of aggregation in the cellular slime mold, *Dictyostelium discoideum*, has been shown to be mediated by way of chemotactic responses to oscillating gradients of cyclic AMP produced by the starving amoebas (*70*).

In studies designed to elucidate the mechanism of chemotaxis in eukaryotes, we have studied chemotaxis using the acellular slime mold, *Physarum polycephalum* (*71*, *72*). After testing a large number of sugars and amino acids we, like Adler (*67*), conclude that the nutritional usefulness of a chemical is not a prerequisite for chemoattraction. However, the nutritional status of the organism influences its responsiveness to attractants. For example, *Physarum* that have fasted respond more quickly to chemotactic agents. There is evidence of a role for cyclic AMP in the process of chemotaxis in *Physarum*. Several components of the cyclic AMP system are present in the slime mold. Both intracellular and extracellular cyclic AMP phosphodiesterase (*73*) have been demonstrated in this organism, as have an unusual cyclic AMP–inhibited protein kinase (*74*) and a particulate adenylate cyclase (*75*). The adenylate cyclase is activated by cyclic AMP itself (*75*), and the nature of this activation appears to be kinetic. Both cyclic AMP and some phosphodiesterase inhibitors, when presented to the slime mold as gradients, will act as attractants to these organisms (*76*). The regulation of adenylate cyclase from *Physarum polycephalum* by the reaction product, cyclic AMP, may have some implications regarding the maintenance of cyclic AMP levels in the slime mold. Such a feedback activation should be consistent with an oscillatory accumulation of cyclic AMP, such as that observed in *Dictyostelium discoideum*. This has already been observed with *Physarum* (*77*). As in the case of *Dictyostelium*, cyclic AMP and its synthesizing enzyme adenylate cyclase, as well as phosphodiesterase, may be integral parts of a communication system in *Physarum*.

Chemotaxis in parasites. Chemotaxis appears to be intricately involved in the completion of the life cycle of parasitic helminths. Migration of trematode miracidia to the intermediary snail host, as well as the attraction of the mammalian host to the motile cercariae of some trematodes, are classical examples of chemotaxis in the life of these parasites. MacInnis (*78*) identified substances from mammalian skin that initiated penetration responses by *S. mansoni* cercariae. Earlier studies by MacInnis and coworkers implicated amino acids and additional compounds as attractants of *S. mansoni* miracidia to the snail host. It has now been demonstrated that snails release substances into water that attract the miracidia. Wright and Ronald (*79*) demonstrated that water conditioned by the snail *Lymnaea palustris* contained amino acids that attract miracidia of *Schistosomatium douthitti*, a blood fluke that infects rodents. MacInnis *et al.* (*80*) have reported that *Biomphalaria glabrata*, the molluscan vector of *S. mansoni*, secretes amino acids into the environment and that these and possibly other substances elicit chemotactic responses by the miracidia of this parasite. The snail-conditioned water not only attracted miracidia but also kept more miracidia in the vicinity of the source of

the stimulus. Furthermore, a mixture of 18 of the amino acids that were identified in the snail-conditioned water was slightly but not significantly more effective than the secretions produced by the snail. It remains to be seen whether any one of these amino acids is more effective than the others in attracting miracidia.

The information gained by such experiments may be useful in developing drugs and methods for controlling bilharziasis and other trematode infections. Analogs of the amino acids secreted by snails may prove to act as antagonists rather than agonists and may result in breaking up an important system for the completion of the life cycle. The incorporation of attractants such as amino acids into larvacides has already been alluded to by Cardarelli (*81*) and MacInnis *et al.* (*80*). Misleading the miracidia to migrate to nonfavorable environments may be sufficient to terminate their life cycle.

Chemotactic research has recently been extended to studies of snail vectors of parasites. Recently, MacInnis' group has shown that fractions of lettuce containing free amino acids are primary attractants for *B. glabrata*, the snail host for *S. mansoni* (*82*). These amino acids have been identified as glutamate and proline. Proline has also been implicated as an attractant of the sea slug *Aplysia* (*83*). Thus it may be possible to devise a trapping mechanism for the snails with these attractants being used as baits.

Conclusions

It is evident that many of the data obtained from studies of the regulatory processes in bacterial and mammalian cells are applicable to processes in parasites. The regulatory processes involving motility and metabolism in parasites may be different from those of their hosts, and it may be possible to exploit these differences in strategies for the chemotherapy of parasites. Studies in this area, however, have more significance than just drug development. Isolation and identification of the different molecular components of these control processes contribute to our basic knowledge within the field of regulatory biology. Some of the basic principles of cell regulation that are shared by the host and parasite may be easier to explore in these lower organisms than in mammals.

Although some investigations of basic processes in parasites are being carried out, the field is still a neglected one. Intensified efforts by molecular biologists, biochemists, and pharmacologists are needed to make a breakthrough in treating parasite helminth diseases. Success in this area is essential if the people of the developing countries are to join the march of progress on an equal footing with the rest of the human race. The high achievement of scientists working against bacterial infections suggests that we can be optimistic about the development of more effective chemotherapeutic agents against parasitic helminths.

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