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EDITORIAL

SOME BIOCHEMICAL EFFECTS OF ANTHELMINTIC DRUGS*

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MOST antibacterial agents interfere with biosynthetic reactions and in this manner inhibit bacterial growth and multiplication. By contrast, most pathogenic forms of helminths (or parasitic worms) susceptible to chemotherapy are the adult, nongrowing stages of the parasite's life cycle. Therefore, biosynthetic reactions proceed at a relatively slow rate in these organisms and for this reason it is not surprising that they are rather refractory to inhibitors of growth. However, these parasites are vulnerable to interference either with mechanisms essential for their motor activity or with reactions providing for the generation of metabolic energy. Studies of the mode of action of anthelmintic drugs have shown that their chemotherapeutic effects can indeed be accounted for in some instances by a depression of muscular activity and in others by inhibition of biochemical mechanisms supplying the energy required for the functional integrity of the parasite.

Like other chemotherapeutic agents, anthelmintics are characterized by their selective toxicity for the parasite as contrasted with their lower host toxicity. In some instances this selective toxicity is based on physiological or biochemical differences between the invading organism and its host. In other cases the oral administration of an anthelmintic compound results in a high drug concentration in the habitat of a given parasitic worm, the intestinal tract, and the low host toxicity can be ascribed to the fact that this drug is not absorbed. A discussion of the effects of several anthelmintics will serve to illustrate these principles.

An example of an anthelmintic drug whose selective toxicity has been demonstrated at the physiological level is supplied by piperazine. Observations of Norton and de Beer[1] on the one hand and of del Castillo[2] on the other, have indicated that the paralysis of the musculature of the common roundworm *Ascaris lumbricoides* produced by piperazine may be brought about either by a neuromuscular blockade or by a hyperpolarization of the muscle. These effects of piperazine are limited to the muscle of the parasite because piperazine has neither myoneural blocking nor hyperpolarizing action on mammalian muscle.[1, 2]

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The paralysis of *Ascaris* muscle produced by piperazine is associated with a marked reduction in the production of succinate by the worm.[3] The drug has no direct inhibitory effect on the metabolic reactions involved in the production of succinate by the parasite.[3] Since the paralysis of *Ascaris* muscle lowers the energy requirement of the tissue, it would appear that the formation of succinate is related to the supply of metabolic energy for muscular contraction.

The habitat of *Ascaris* and of many other adult forms of helminths is the intestinal lumen where the oxygen tension is low. These conditions can account for the fact that fermentation of carbohydrate, rather than aerobic metabolism, provides the major source of energy for intestinal helminths. In contrast to the tissues of their hosts, succinate rather than lactate is a major fermentation product of these helminths.[4] The anaerobic formation of succinate in *Ascaris* and in the cestode *Hymenolepis diminuta* can be accounted for by the following reaction sequences: CO_2 is fixed into phosphoenolpyruvate (PEP) produced during glycolysis. This reaction results in the formation of oxalacetate (OAA) and is catalyzed by the action of PEP carboxykinase.[5, 6] Reduction of OAA to malate is catalyzed by malic dehydrogenase.[5] Fumarase catalyzes the dehydration of malate to fumarate.[7] In *Ascaris* mitochondria an electron transport system is present which catalyzes the reduction of fumarate by reduced nicotinamide adenine nucleotide (NADH).[8] Evidence is available which indicates that in this system the anaerobic reduction of a flavoprotein by NADH is coupled with the phosphorylation of adenosine diphosphate (ADP).[9, 10] Therefore, energy is generated in the form of adenosine triphosphate (ATP) under anaerobic conditions at the electron transport level. For this reason, succinate fermentation is of physiological advantage for the parasite when compared with lactate fermentation.

The significance of succinate fermentation as a source of energy for the cestode *H. diminuta* has become evident during studies concerned with the mode of action of anticestodal agents. Fumarate is required as the terminal electron acceptor of the ATP generating mitochondrial electron transport system. The formation of this C_4-dicarboxylic acid is dependent on the fixation of CO_2 into PEP. When this is prevented by incubation of the tapeworm in the absence of CO_2, a marked decrease in the ATP levels of the worm is observed.[11] This is reversed on readmission of CO_2. Similarly, CO_2 is required for the optimal turnover of phosphate in ATP. After incubation of the worms in the presence of radioactive inorganic phosphate for 10 min, the specific activity of the ATP isolated from the organism is much lower when CO_2 is excluded.[11] Therefore, in the intact worm, inhibition of fumarate formation by the omission of CO_2 results in an inhibition of the anaerobic generation of ATP.

Further evidence for the occurrence of mitochondrial phosphorylations in the intact worm under anaerobic conditions has been obtained by the use of two known uncouplers of oxidative phosphorylations, dinitrophenol (DNP) and carbonyl cyanide chlorphenylhydrazone. If *H. diminuta* is incubated anaerobically in an atmosphere containing 95% N_2 and 5% CO_2, low concentrations of these compounds markedly inhibit the incorporation of ^{32}P-labeled inorganic phosphate into ATP.[11] It is of interest that several anticestodal agents have a similar action. Among them are two compounds which now are considered as the most effective and the least toxic available chemotherapeutic agents used in the treatment of tapeworm infections, dichlorsalicylamide ('Yomesan') and dichlorophen. Another anticestodal compound is desaspidine, a phloroglucinol derivative which is the active principle of oleoresin of

aspidium. These three compounds have some structural similarity with DNP; they are chloro-, nitro- and methoxy-substituted derivatives of phenol. Furthermore, both desaspidine and chlorsalicylamide have been reported to uncouple oxidative phosphorylations in liver mitochondria.[12, 13] In the presence of low concentrations of these compounds, the turnover of ^{32}P-labeled inorganic phosphate in ATP is reduced during anaerobic incubation of *H. diminuta*.[11] It appears that this inhibition occurs at the level of the ATP–P_i exchange reaction because these compounds markedly reduce the ATP–P_i exchange catalyzed by isolated tapeworm mitochondria.[11] An inhibition of this exchange reaction, which is considered to be one of the steps involved in phosphorylations during the electron transport from NADH to flavoprotein, could account for the mode of the anticestodal action of these compounds. The low toxicity of chlorsalicylamide and of dichlorophen probably could be ascribed to the fact that they are not absorbed from the gastrointestinal tract.

Before turning our attention to the biochemical effects of antischistosomal drugs, it should be pointed out that venous blood, the habitat of adult schistosomes, has a much higher oxygen tension than the physiological environment of intestinal helminths; yet, as in the case of the latter parasites, survival and reproduction of schistosomes depend predominantly, if not exclusively, on the anaerobic utilization of carbohydrate.[14, 15] On the other hand, in contrast to intestinal helminths, schistosomes are homolactic fermenters because they convert carbohydrate nearly quantitatively to lactic acid rather than to succinate.[16] In intestinal helminths, the reaction which accounts for the formation of C_4-dicarboxylic acids is catalyzed by PEP carboxykinase.[5] However, PEP, the substrate of this reaction, can be removed by the action of pyruvate kinase which catalyzes the dephosphorylation of PEP to pyruvate in the presence of ADP. There are marked differences in the pyruvate kinase activities of helminths.[5] Pyruvate kinase activity is very high in *S. mansoni*, whose major, if not exclusive, product of carbohydrate metabolism is lactate. It is about 300 times lower in *Ascaris*, which produces virtually no lactic acid. Very low pyruvate kinase activities have been reported also in the third larval stages of the nematode *H. contortus* by Ward *et al.*[17] A somewhat intermediate activity of this enzyme is found in the tapeworm *H. diminuta*, whose major fermentation product is succinate, but which, in contrast to *Ascaris*, produces some lactate. When the pyruvate kinase activities of these three helminths are compared with those of PEP carboxykinase, pyruvate kinase activity is ten times greater in *S. mansoni*, suggesting that the metabolism of PEP is directed toward dephosphorylation in this homolactic organism. By contrast, in *Ascaris* muscle, pyruvate kinase activity is 25 times lower than PEP carboxykinase activity, thereby providing conditions strongly favoring OAA formation. The predominance of succinate over lactate formation in the tapeworm *H. diminuta* is reflected in a higher activity of PEP carboxykinase over pyruvate kinase.[5] Therefore, in these helminths, fermentation in the direction of either succinate or lactate may be controlled by the competing activities of these two enzymes.

The oldest chemotherapeutic agents used in the treatment of schistosomiasis are the trivalent organic derivatives of antimony. The evidence that these compounds exert their antischistosomal action by inhibiting the activity of a single enzyme of the parasite, i.e. phosphofructokinase (PFK),[18, 19] can be summarized as follows: (1) The concentrations of antimonials which produce a nearly complete inhibition of the PFK activity of *S. mansoni in vitro* are of the same order of magnitude as those

prevailing in the parasite after administration of chemotherapeutically active doses of these drugs to the host. (2) In extracts of schistosomes, the activity of PFK controls the rate of glycolysis which, in turn, supplies the major source of energy to the parasite. (3) After the administration of subcurative doses of antimonials, the substrate of the PFK reaction, fructose 6-phosphate (F6P), accumulates while the concentration of the product, fructose-1,6-diphosphate (FDP), is markedly reduced in the worm, indicating that under these conditions the activity of this enzyme is inhibited *in vivo*.

The inhibition of schistosome PFK activity is competitive with and reversible by F6P, one of the substrates of the enzyme.[18] Therefore, it appears that antimonials exert their inhibitory effect either by a competition with F6P for the same site of the enzyme and a displacement of the substrate from this site, or F6P alters the configuration of schistosome PFK in such a manner as to render it less susceptible to the inhibitory action of antimonials.

The reversibility of the inhibitory effect of antimonials on schistosome PFK is reflected also in the time course of the chemotherapeutic actions of these compounds *in vivo*. Buttle and Khayyal[20] have found that 2 hr after a single intraperitoneal injection of potassium antimony tartrate to mice infected with *S. mansoni*, the worms lose their attachment to the mesenteric veins and are carried to the liver sinuses by the venous blood flow. However, unless administration of the antimonial is continued, nearly all the schistosomes have returned to the mesenteric veins after several days. When 2 hr after the injection of the antimonial, nearly all the worms have shifted to the liver, there is an accumulation of F6P and a marked decrease in the concentration of FDP.[18] Therefore, this shift is associated with an inhibition of PFK activity in the worms. Furthermore, the recovery of the worms and their return to the mesenteric veins coincide with a reversal of the inhibition of PFK activity because the concentrations of the hexosephosphate esters have returned to their control levels.[18] This close association between chemotherapeutic effects and reversible biochemical changes supplies further evidence for the causal relationship between inhibition of PFK activity and the antischistosomal action of trivalenlt organic antimonials.

As a result of PFK inhibition, the ratio between the sum of hexosemonophosphates (F6P + G6P) and that of FDP and triosephosphates is markedly increased. This indeed is the case in worms which have shifted to the liver shortly after the administration of an antimonial. However, the shift that follows the administration of another antischistosomal drug, niridazole, or of a barbiturate is not associated with a change in this ratio, indicating that the hepatic shift produced by these nonantimonial drugs is brought about by mechanisms other than inhibition of PFK activity. Therefore, the changes in the phosphate ester levels are a specific effect of antimonials and are not observed after the hepatic shift produced by other compounds.

It has been assumed that the chemotherapeutic actions of antimonials are brought about by inactivation of sulfhydryl (SH) enzymes of the parasite.[21] While schistosome PFK is activated markedly by SH compounds (such as cysteine, glutathione, penicillamine or mercaptoethanol), the inhibitory effect of antimonials is not reduced in the presence of such SH compounds; in fact, it is somewhat greater.[18] Therefore, inactivation of SH groups of PFK is not involved in this inhibition of enzyme activity by antimonials.

Many attempts have been made to develop an antischistosomal agent which is less toxic than are antimonials and which can be administered orally. One of them is a

carbonium derivative, Tris (*p*-aminophenyl) carbonium chloride (*p*-rosaniline).[22] Administration of this compound to the host produces a paralysis of the acetabulum, the muscular organ with which the worm attaches itself to the internal wall of a mesenteric vein. This paralysis is reversed *in vitro* by certain cholinergic blocking agents such as atropine and by a ganglionic blocking agent, mecamylamine.[23] The musculature of schistosomes responds to acetylcholine (ACh) with a depression of muscular activity.[24] Therefore, the reversal of a paralysis by a cholinergic blocking agent suggests an accumulation of endogenous ACh brought about by an inhibition of acetylcholinesterase (AChE) activity. By the use of the modified histochemical method of Koelle and Friedenwald,[25] it has been found that in schistosomes AChE is localized in the central ganglia (connected by a commissure), the nerve trunks and the acetabulum.[23] In worms removed from animals which had received subcurative doses of *p*-rosaniline, a very marked decrease in the activity of AChE is observable in the nervous system and in the acetabulum of the worm.[23] Therefore, schistosomes appear to be susceptible to inhibition of AChE activity.

The most recent antischistosomal agent is a nitrothiazole derivative, 1-(5 nitro-2 thiazolyl)-2 imidazolidinone (niridazole).[26] Like *p*-rosaniline and unlike trivalent antimonials, niridazole is a relatively slow-acting drug; a shift of the worms toward the liver begins only 4 days after the oral administration of the drug has been initiated. As pointed out above, under these conditions there is no evidence for an inhibition of PFK activity in the worms. Also, AChE activity is indistinguishable from that of the controls. Therefore, niridazole must have antischistosomal effects which are distinctly different from those caused by *p*-rosaniline or by antimonials. Niridazole produces two types of changes in the parasites prior to their shift to the liver. At 24–48 hr after the first dose, changes in the female reproductive system are already observable, when the worms are incubated with a tetrazolium salt which can serve as an *intra vitam* stain for this organism.[23] Administration of niridazole to the host results in the swelling and distortion of the ovarian cells. Furthermore, stained vitelline material appears in the egg after treatment, indicating a defect in, or the absence of the egg membrane, which is impermeable before treatment. These changes are not specific for niridazole and are indistinguishable from those observed after administration of other antischistosomal drugs such as *p*-rosaniline or trivalent antimonials.[23] Therefore, it appears that interference with any physiological or biochemical mechanism which supplies energy for the biosynthetic activities of the female reproductive system can bring about its functional impairment.

Another change produced by the administration of niridazole to the host consists in a reduction of the glycogen stores of the male worms. The degree of the glycogen loss is dependent on the dose. Glycogen loss precedes the hepatic shift because the glycogen levels of the worms are decreased even after the administration of doses which are lower than those (i.e. 100–200 mg/kg) required to cause a loss of attachment of the parasite to the mesenteric veins. Moreover, 24 hr after the oral administration of a single dose of 200 mg per kg of this compound, there is already a significant decrease in the glycogen concentration.

A decrease in the glycogen stores of schistosomes produced by the administration of nitidazole could be brought about either by an inhibition of glycogen synthesis or by an increased rate of glycogenolysis. When a dosage schedule of niridazole is used which results in a marked glycogen loss of the worms, no changes in the activities

of the parasite's glucose 6-phosphate independent and dependent uridine diphosphate glucose–glycogen transferase activities are detectable. Accordingly, the possibility of an activation of glycogenolysis has been considered. *S. mansoni* has high glycogen phosphorylase activity, which rapidly disappears on incubation of schistosome extracts at 30° when enzymatic activity is determined in the absence of adenosine 5′-monophosphate. Phosphorylase activity is restored within a few minutes when ATP and Mg^{++} are added to these extracts. Furthermore, glycogen phosphorylase inactivation is prevented in the presence of NaF (0·02 M). These observations suggest the presence in the worms of an active and of an inactive glycogen phosphorylase and the interconversion of these two forms by a phosphorylase kinase and by a phosphorylase phosphatase. The rate of inactivation of active phosphorylase, presumably by a phosphorylase phosphatase, is reduced in extracts of schistosomes after the administration of niridazole to the host. Again there is a dose–effect relationship, i.e. the higher the dose the greater is the inhibition of phosphorylase inactivation. This effect of the drug coincides with or precedes the decrease in the glycogen concentration of the worm. For example, inhibition of phosphorylase inactivation is already detectable 12 hr after administration of the drug, while a significant decrease in the glycogen content is observed after 20 hr. Inhibition of phosphorylase inactivation produced by niridazole results in the availability of more active phosphorylase, which catalyzes the degradation of glycogen and thereby could account for the observed reduction in the glycogen stores of the worms.

Inactivation of schistosome phosphorylase is observed not only after the administration of subcurative doses of niridazole to the host, but also after incubation of the worms with the drug *in vitro*. However, the concentrations required to produce this effect *in vitro* are somewhat higher than those prevailing in portal venous blood after its administration to the host.[27] Therefore, the possibility must be considered that a metabolite of niridazole is responsible for the antischistosomal actions of this drug. It has been found by Faigle and Keberle[27] that 24 hr after the administration of a single dose of niridazole only its as yet unidentified metabolites, but not the drug itself, can be found in the tissues of the host and of the parasite. Yet, the inhibition of phosphorylase inactivation progressively becomes more pronounced over a period of at least 72 hr under these conditions. The antischistosomal activity of one or several metabolites of niridazole produced by the host or by the parasite (or both) could explain such a time course.

The diversity of the selective biochemical effects produced by anthelmintic drugs suggests that a better understanding of the relationship between the biochemical actions of these compounds and their chemotherapeutic action can reveal more mechanisms which are essential for the functional integrity of the parasite and which are vulnerable to inhibition by chemical agents. This, in turn, can provide one of the prerequisites for the rational design of chemotherapeutic agents.

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