A REVIEW OF PHARMACOLOGY:

SPECIAL REFERENCE TO

ANTHELMINTICS

By

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CHAPTER I

INTRODUCTION

Pharmacology is the science that deals with the effect of chemical agents upon living cells. The history of pharmacology as a discipline goes back as far as the primitive people who discovered the relationship between certain plants and their affect on certain disease states. This period of ancient pharmacology was characterized by empiric observations in the use of crude drug preparations. Today's period of modern pharmacology is based on experimental investigations that are designed to show the site and mode of action of chemical agents, i.e., drugs. These chemical agents may occur naturally within the cell's environment or may be a synthetic component of the cell's environment. The primary concern of the pharmacologist is the effect of these agents on metabolic processes of organisms. This is most often achieved by studying these aspects in experimental animals and then applying what is learned from the studies to the use of the drug in man. Because of this comparative nature of studying the affects of drugs, be they natural or synthetic compounds, this area of pharmacology is often referred to as comparative pharmacology.

The tremendous gains that have been made most recently in pharmacology are due primarily to the developments of other basic sciences that have enhanced our understanding of the basic framework by which organisms exist. Most often when a drug is being studied, valuable

contributions come from cell biologists, biochemists, and pathalogists as well as many others. The contributions of these scientists allow for an enhanced knowledge of the pharmacodynamics of the drug. This area of pharmacodynamics deals with how a drug is affecting an organism or cell at the biochemical and physiological level. Any study of this type requires knowledge of how the drug is absorbed, distributed, metabolized and excreted by the organism. The evidence from these studies indicates that there is a strong correlation between the chemical structure of the agent and the effects it elicits. With this type of information, an organic chemist is able to design a drug with predictable actions. The final goal of these chemists is to produce organic compounds that have a high specificity for the invading organism with little or no effect on the host.

This concept of high specificity for the invading organism and low specificity for the host is very important in pharmacology. Unfortunately, this is not the case for most of the drugs employed in modern therapeutics. For this reason there is a division of pharmacology that deals with the toxicology of drugs. This area of pharmacology that works to control the toxicities associated with the administration of therapeutic agents is one that cannot be taken lightly. Through the use of comparative pharmacology, it is possible to determine the toxic effects of drugs on experimental animals and then to predict how the drug will affect the host upon administration of the drug. By changing the substituents present on the basic drug molecule and studying the variance of effects accompanying each of these, it is possible to isolate those chemical substituents that are toxic to the hosts. This type of information is a valuable tool to the organic chemist trying to

synthesize drugs with low toxicities in the host. Despite all the work that has been done in this area, there are still many drugs used therapeutically whose toxicities cannot be altered without a concomitant loss of therapeutic effectiveness. For this reason it is imperative that anyone dispensing or using drugs be aware of their potential danger and the signs associated with the toxic affects of the drug. Meanwhile much research will continue in the field of pharmacology so that one day it may be possible to alleviate the adverse reactions accompanying many of these drugs.

Absorption, Distribution and Excretion of Drugs

Perhaps the most important characteristic associated with any drug is how it is absorbed from the site of administration. The bodies of man and animal have within them a number of permeability barriers. How the drug crosses these barriers determines how effective the drug will be against an invading organism. Extensive research in pharmacodynamics has shown that the absorbance of a drug is dependent on conditions of pH, solubility of the drug in solution, solubility of the drug in lipid, and the amount of the drug present at the site of absorption.

Drugs exist as either organic acids or organic bases. As a result they have pK values associated with them. If the pH of the solution the drug is dissolved in is below that of the pK value, the drug will be primarily in the nonionized form. If the pH of the solution is higher than that of the pK value, the drug will be primarily in the ionized form (13, 19, 1). Due to the characteristics of absorptive membranes only the nonionized fraction of the drug will pass through the membrane

if it is soluble in lipid. For this reason it is the nonionized, lipid soluble fraction of the drug that represents the active moiety of the drug.

There are three ways the drug can cross the absorbing membrane. First, the lipid soluble nonionized form of the drug may simply diffuse across the membrane (13, 1, 25). Second, the active form of the drug may be "carried" from one side of the membrane to the other. Thirdly, the drug moieties may pass through pores within the membrane structure (12). The absorption mechanisms involving diffusion of the lipid soluble fraction of drug and the passage of drug moieties through pores of the membrane respond to a concentration gradient. That is, the drug form will move in a direction of high concentration to a lower concentration of itself. The absorption mechanism involving a carrier associated with the membrane may or may not respond to a concentration gradient. If the carrier responds to a concentration gradient across the membrane, carrying the active moiety of the drug from a region that has less of this drug form present, then the absorption mechanism is termed facilitated diffusion (22). On the other hand, if the carrier is concentrating the active moiety on one side of the membrane, i.e., is carrying the drug form to a region that has a greater number of the transported drug form, then the absorption mechanism is termed active transport (16). To determine by which absorptive mechanism a drug crosses the absorptive membrane, an analysis of the kinetics of absorption must be performed. Studies of this type surpass the scope of this paper, but nevertheless the knowledge of how the active moiety of the drug crosses the absorptive membrane is important when considering how much of a drug should be given to be therapeutic.

If the pH determines what state the drug predominates in solution according to the pK value of the drug and for all therapeutic purposes the nonionized fraction of the drug is the only active form of the drug it is logical to choose the drug of choice by matching it with the pH of the solution it will be dissolved in. All that is needed is a drug with a pKa value that corresponds favorably to the pH of the fluid surrounding it. This usually leads to complicated calculations resulting in higher doses of the drug or an addition of agents that change the pH of surrounding media (13). One other factor that will influence the concentration gradient at the absorptive site is the pH gradient that exists between the fluid at the absorptive surface and the plasma (1, 13, 17).

Many times the ratio of ionized form of drug to nonionized form of drug will not be the same when comparing drug forms present in gastrointestinal secretions versus those of the plasma. A change of this type is going to affect the amount of drug administered and the eventual distribution of drug throughout all body fluids. Once the drug is administered and the nonionized fraction of drug reaches the plasma, the establishment of an equilibrium of this drug form with all body fluids is begun. This equilibrium of the nonionized fraction of drug is going to be dependent on several factors. For instance, it has been shown that some drugs have high affinities for certain tissues of the body, i.e., liver, kidney, adipose tissues (13). This accumulation of drug within a tissue type will affect the amount of nonionized drug available for equilibrium. Another factor altering the available equilibrating moiety will be the degree of binding to plasma proteins. It is imperative that the amount of drug given make up for the presence of active

drug moieties in tissues of high affinity, and the binding of drug to plasma proteins as well as a knowledge of how each of these will respond to shifts in equilibrium as the drug becomes inactive.

Shifts in the equilibrium concerning the free nonionized fraction of drug may result from the excretion of drug or drug metabolites from the body. The method by which a drug will be excreted from the body varies with each drug. The organs associated with excretion of most drugs is the kidney and liver (13). Excretion by the kidney results from the ability of the kidney to filter the drug moieties from the plasma passing through the glomerular network of the kidney nephron (13, 11). If the drug is such that it will not be reabsorbed through the renal tubular walls, then the drug passes out of the body in the same form that it existed in the plasma. However, many times the drug must be altered in some way so that eventually the body will be ridded of the drug. The other primary mechanism of ridding the body of the drug is hepatic excretion. Many drug metabolites are formed in the liver and excreted in the bile, which passes out of the intestinal tract as a component of fecal material (13).

Secondary methods of excretion which facilitate the removal of drug from the body include various glands and their secretions. Examples of these encompass the salivary glands, sweat glands and the mammary glands of the female (13). The only form of drug that is excreted by these glands is the lipid soluble, nonionized moiety of a drug. Excretion of drug from the sweat gland is of little concern in most cases. However, excretion of this drug form in secretions of either the salivary glands or the mammary gland do deserve consideration. In the case of salivary excretion, the nonionized, lipid soluble fraction of the

drug will be excreted into the mouth and then swallowed putting it back into the stomach where it may be reabsorbed into the plasma. Recycling of the drug in this fashion will have an effect on the duration of action of the drug and should be a concern to those administering the drug. Excretion of the lipid soluable, nonionized fraction of the drug through the secretions of the mammary gland is a major concern to those administering the drug as well as for those who will consume the milk obtained from the gland. Because of the possibility for excreting the active drug form into the milk, it is often stressed that drugs not be given to females producing milk that will be consumed.

Biotransformation of Drugs

The metabolism of a drug plays an important role in its therapeutic activity and toxicity (39). Detoxification of a drug refers to the changing of an active form of drug to an inactive form. This inactive form is the metabolite of the drug and is the result of biotransformation by enzymes. The mechanism of biotransformation involves enzyme systems that change the structure of the drug so that the resulting compound is more readily excreted from the body or has a different biological activity. Increasing the polarity of a compound is often the objective of biotransformation but sometimes, biotransformation leads to a change in structure that only alters biological activity doing nothing to enhance the excretion of the drug. Transformation of this latter type often lead to an explanation of the side affects or after affects associated with various drugs.

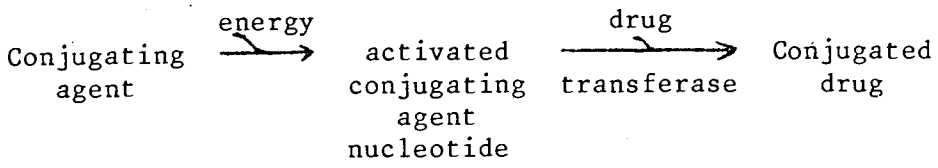
Mechanisms of biotransformation involve Phase I reactions that oxidize, reduce, or hydrolize, and Phase II reactions that lead to

conjugation of the drug so that a different compound results (17, 39). The enzymes catalizing both these types of reactions are found in association with various tissues (13). The amount of variance associated with these enzymes in different species often explains the differences in drug action as seen from species to species (12, 36). If comparative studies in pharmacology are to tell us anything about the use of a drug in man, then the enzyme systems of man and the experimental animal must be very similar to each other.

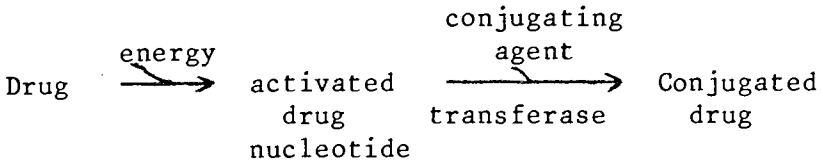
The biotransformations that involve oxidation, reduction, and hydrolysis of the drug often involve enzymes of the normal metabolic pathways occurring within the liver microsomes (39). The number of oxidations possible are quite numerous and may include such things as aromatic hydroxylation, N-oxidations, sulfoxidations, or aliphatic hydroxylation (14, 39). Reductions may be a nitroreduction, azoreduction, or an alchohol dehydrogenation (14). The hydrolysis reactions are like those involved in the metabolism of procaine resulting in the production of p-aminobenzoic acid and diethylaminoethanol (14). The hydrolysis reactions are like those involved in the metabolism of procaine resulting in the production of p-aminobenzoic acid and diethylaminoethanol (14).

The mechanisms involving Phase II reactions that result in the conjugation of the drug to some other chemical moiety are generally more complex than the Phase I reactions. Phase II reactions are catalyzed by enzyme systems that seem to be primarily concerned with compounds the body regards as foreign (39). These reactions require some kind of "active" intermediate compound and a transferring enzyme. On the one hand the conjugating agent forms an active intermediate that is then

combined with the drug through transferase activity. In other cases, the drug may become activated and then combine with the conjugating agent via the transferase. These two schemes are illustrated by Williams (39) as follows:



and



Any drug that has a glucuronate metabolite, follows the scheme involving a conjugating agent, glucuronic acid, an active in intermediate, uridine diphosphate glucuronic acid, and a glucuronyl transferase. An example of the second scheme of conjugation is the formation of hippuric acid from benzoic acid through benzoyl-CoA, the activated drug nucleotide. For this reaction the transferase is known as glycine N-acylase. The variation associated with different species in their ability to carry out these schemes is probably due to the individual species ability to synthesize the necessary active intermediate nucleotides, the presence of transferring enzymes, or the availability of the conjugating agent within the species. An understanding of these differences reinforces the concept that comparative studies must be done so that the metabolic systems of the experimental animal will be very similar to the systems of the species to which the drug will be given therapeutically.

Mechanisms of Drug Action

Comparative studies of drug behavior are valuable in that they may

provide insight as to how the drug is working on metabolic systems of the organism given the drug. Studies of this nature that employ techniques that allow for the measurement of certain parameters and how they change with administration of a drug or similar compounds, may lead to the isolation of area of interest. These areas of interest usually involve the site of drug action at the cellular level as well as which of the constituents on the drug is responsible for the drug's action. Despite the importance of this type of information, analyses of this type are very difficult and time-consuming which keeps many researchers in other areas as well as those in this field slow in producing data.

It is clear to those investigating this field of how and why drugs work that drugs taken to fight the invasion of certain organisms are usually highly specific for the invader as constrasted by a low toxicity to the host (30). This suggests that the mechanism of drug action involves biochemical differences between the host and the invading organism. These biochemical differences should be the result of differences in the metabolic systems of the two or of differences in nucleic acid composition and sequence that determine the structures of proteins and enzymes found within each of the organisms (5). Knowledge of the nature of these differences provides valuable information to those designing the structure of drugs that will be selective chemotherapeutic agents against invading organisms.

Ways of isolating the areas suspected of being altered by a chemotherapeutic agent are numerous. There are certain techniques that seem to be very popular with those investigating how these biochemical differences respond to the application of drug. These techiques may involve an examination of how metabolic pathways are altered or how the

activity of the Na/K-ATPase dependent ion transport pump is affected.

Much of the data gathered thus far seems to indicate that a large number of drugs inhibit enzymes involved in carbohydrate metabolism (reviewed by Beames [2], Cohen [5], and Mansour [21]). The determination of how a drug effects this metabolic pathway involves measuring the uptake and utilization of carbon sources, the presence of metabolic intermediates as well as the end products to be excreted. By measuring the occurance of the compounds in a control animal and then comparing these results to those obtained in the same fashion from an animal who had been given the drug of interest, it becomes possible to isolate points along the metabolic pathway that are altered by the presence of the drug. Once these points have been determined with some degree of certainty, the enzyme involved in the drug reaction can be isolated and collected for further study. Studies of this type not only help in the understanding of drug action, but also aid our understanding of metabolic systems found in lower life forms.

Many times these studies designed to measure the concentrations of metabolic substrates, intermediates, and end products do not always give an indication of the exact mechanism of action involved. Many of the enzymes involved in metabolic pathways respond to the internal conditions of the cell (15). Consequently, any agent that disrupts the mechanisms involved in maintaining intracellular homeostasis will result in the breakdown of metabolic pathways. The Na/K-ATPase dependent ion transport pump is one of the mechanisms involved in homeostasis of cells due to the fact that this pump maintains the concentration gradient of ions necessary for normal enzymatic behavior. Interfering with the functional capacities of this pump leads to the

eventual death of the cell.

By measuring the intracellular concentration of an ion maintained by this active pump and noting any changes that occur in response to the exposure of a drug, a statement about how and where the drug acts may be made (15, 19, 30). One of the techniques designed for the analysis of activity associated with this pump involves a study of ionic flux values associated with the cells of a tissue. A more complex technique involves intracellular measurements obtained from micropuncture of an individual cell. In each of these techniques, there is a control animal and an experimental animal, to which the drug of interest is given, so that differences in ionic concentrations that occur with the passage of time or changes in pH will not come into play when analyzing the data for significant differences due to the drug's effect.

In flux studies, the usual procedure involves some radioactive ion whose flux is determined by the Na/K-ATPase dependent ion transport pump (15, 30). This ion must be one that is nondistinguishable by the membrane in either its normal state or the radioactive state. The design of these flux studies is such that the uptake of this radioactive ion is correlated to the passages of time. This is achieved by taking segments of a tissue that are equal in weight and in their origin (all the segments used in these studies must be from the same place in the animal). All of these alike segments are then placed in solutions containing the radioactive ion (isotope). At certain time intervals the tissues are removed from the solution for analysis. The studies using tissue from the control animal will show the normal uptake of the isotope while tissue from the experimental animal will show how this uptake has been affected by the addition of the drug to the tissue.

By comparing the data and looking for significant differences between the two conditions, it becomes possible to determine if this pump is the site of drug action.

In the above flux studies, the resulting data showed how much of an ion was taken up by a collection of cells but says nothing about what is happening within each of these cells. If we can measure the intracellular ionic activities, then we can make a statement about the concentration and electrochemical gradients that result from the metabolic activities of the living system (19). One of the best ways to do this is to fabricate a glass electrode that has a tip diameter of 5 to 10 microns and an electrical resistance of 8 to 13 megaohms. A microelectrode fashioned in this way can penetrate a single cell without significant damage to the cell membrane.

The design of this technique involves a complicated set-up where the tissue being investigated is suspended in a chamber of saline compatible with the tissue and the microelectrode is connected to a system of amplifiers and chart recorders. Usually the microelectrode is mounted to micromanipulator to facilitate accurate impalement of the cell with the microelectrode (18, 19). By operating the micromanipulator it is possible to make several impalements of absorptive epithelial cells and to record signals from the microelectrode that are due to gradients across the cell membrane.

The way in which the microelectrodes respond to the gradients involves a transfer of charge between a metal wire and an electrolyte solution at its interface (10). Once the microelectrode enters a solution of ions, there is a tendency for ions to move into or out of the microelectrode. Movement of this type alters the equilibrium

existing at the metal/electrolyte interface. The magnitude of this shift is the signal that passes through an amplifier to give the final recorded signal (system reviewed in Nastuk 23). The recorded signal represents a potential difference between the microelectrode and some reference point in the solution (19). If the microelectrode is inserted into a solution that is separated from another solution by a membrane then the microelectrode will measure the potential difference across this membrane that is a function of the ionic distribution on either side of the membrane (16, 19). Unfortunately, measurements obtained from this microelectrode say nothing about the ions involved in the establishment of this potential difference. For specific ion analysis it is necessary to fill the tip of the microelectrode with an organic electrolyte having a high selectivity for a given ionic species (18). The procedure involving these ion specific microelectrodes is different from the aforementioned procedure involving reference electrodes.

When employing microelectrodes that are specific for an ion, it is necessary to standardize the electrode before its use in biological systems. This standardization procedure involves exposing the electrode to solutions of various concentrations of the ion exchanged by the resin within the tip of the microelectrode. The result of this exposure of the microelectrode to different concentrations of the ion is a calibration curve that can be applied in the conversion of experimentally obtained potential measurements from this electrode to the concentration gradient it represents. Only after this curve is constructed will the data obtained in the impalement of biological cells be understood. It should be pointed out that these ion specific electrodes respond to the electrochemical gradient as well as the ionic concentration gradient.

For this reason it is necessary that the membrane potential measured by the reference electrode be subtracted from the final recorded signal obtained from the ion specific electrode if the actual potential difference between the ion specific electrode in the cytoplasm of the cell and the reference point in the bathing media is to be known (19).

Both the flux studies and those of micropuncture designed to show whether or not the drug is interfering with the activity of the NA/K-ATPase active pump can be compared to those studies involving metabolic inhibitors effects on this pump (38, 40). After all, if the drug is interfering with the pump's ability to work, then it should be considered to be a metabolic inhibitor. Therefore, the changes in intracellular ionic concentrations or uptake of radioactive isotopes that occur in tissues exposed to known metabolic inhibitors should be representative of the kind of data seen in studies designed to identify agents of this type. By reviewing the literature on metabolic inhibitors and obtaining the above information as well as the chemical structure of these inhibitors, it is possible to analyze the presence or absence of similarities between the drug in question and a metabolic inhibitor which eventually leads to an idea of how the drug is acting.

The experimental procedures mentioned thus far represent only a few of the many ways to determine mechanisms by which drugs act. Any technique that can measure differences occurring within a system in response to the passage of time or the presence of drug will be effective. Many times the type of technique employed is chosen in light of the type of equipment available to the laboratory performing the experiment. Other times the procedures involved in interpreting the data and the reliability of the data will determine the procedure used.

In any case, a brief review of the literature will provide information necessary for deciding which of the techniques is best suited for your purposes.

CHAPTER II

INTRODUCTION TO HELMINTHS

Helminth is a general term that refers to several phyla of worms most of which are parasitic in nature. Helminthiasis refers to the diseased condition brought on by the presence of worms in the body of a suitable host. Throughout the history of man these parasites have been a major etiological agent of infection (8). There is not a nation in this world whose inhabitants are not experiencing the problems associated with helminthiasis. Helminths invade the gastrointestinal tract, the circulatory system and the muscle systems of their hosts. They constitute a continual drain on human energy resources that result in hindered economic growth.

Helminthiasis usually results after exposing the egg or larval form of the worm to certain tissues of the body. The most common of these are the tissues of the mouth and feet. Once the helminth has invaded the body of its host, a general wasting away of the body occurs in conjunction with feelings of nausea, dizziness, exhaustion and abdominal discomfort. Because of the prevalence of this condition throughout the world and the amount of damage these parasites inflict on their hosts, the designing of drugs effective against these organisms has become the main concern of many pharmaceutical engineers.

One factor that for many years hindered the development of chemotherapeutic agents against helminths was a lack of understanding of the

biochemistry and physiology of the helminths. Although the evidence gathered at this point does not give a complete picture of how helminths "work", it does provide enough information that aids in the development of drugs against these organisms. This information came from studies that indicated a divergency of the helminth's metabolic pathways from those of its host (21, 27, 28, 29, 30, 33). The divergent pathway that is of greatest interest is that of carbohydrate metabolism.

In both host and parasite the catabolism of carbohydrates provides the high energy levels necessary for the survival of the organisms. This utilization of "food" begins with the phosphorylation of glucose (2, 22, 29, 33). The phosphorylated glucose molecule is then acted upon by a number of glycolytic enzymes and the result is the conversion of glucose into two molecules of phosphoenolpyruvate. In the host tissues phosphoenolpyruvate is formed into pyruvate by the enzyme pyruvate kinase and the reaction produces a nucleotide triphosphate (ATP). This enzyme is not functional in many of the intestinal helminths (29, 33).

Biochemists have analyzed the tissues of many helminths for the presence of glycolytic enzymes. What they found is that helminths catabolize glucose via the glycolytic pathway up to the point of phosphoenolpyruvate formation (2, 28, 29, 33). The metabolic pathways that have been described for the tissues of Ascaris suum are represented in Figure 1.

In helminths the reducing agent of this metabolic pathway is NADH (29). Because of the reduced activity of pyruvate kinase in helminths (Figure 1-C) phosphoenolpyruvate (PEP) is most often reduced by NADH and then subsequently combined with CO_2 by the enzyme PEP carboxykinase (Figure 1-D) (29, 33). The oxaloacetate that results eventually forms

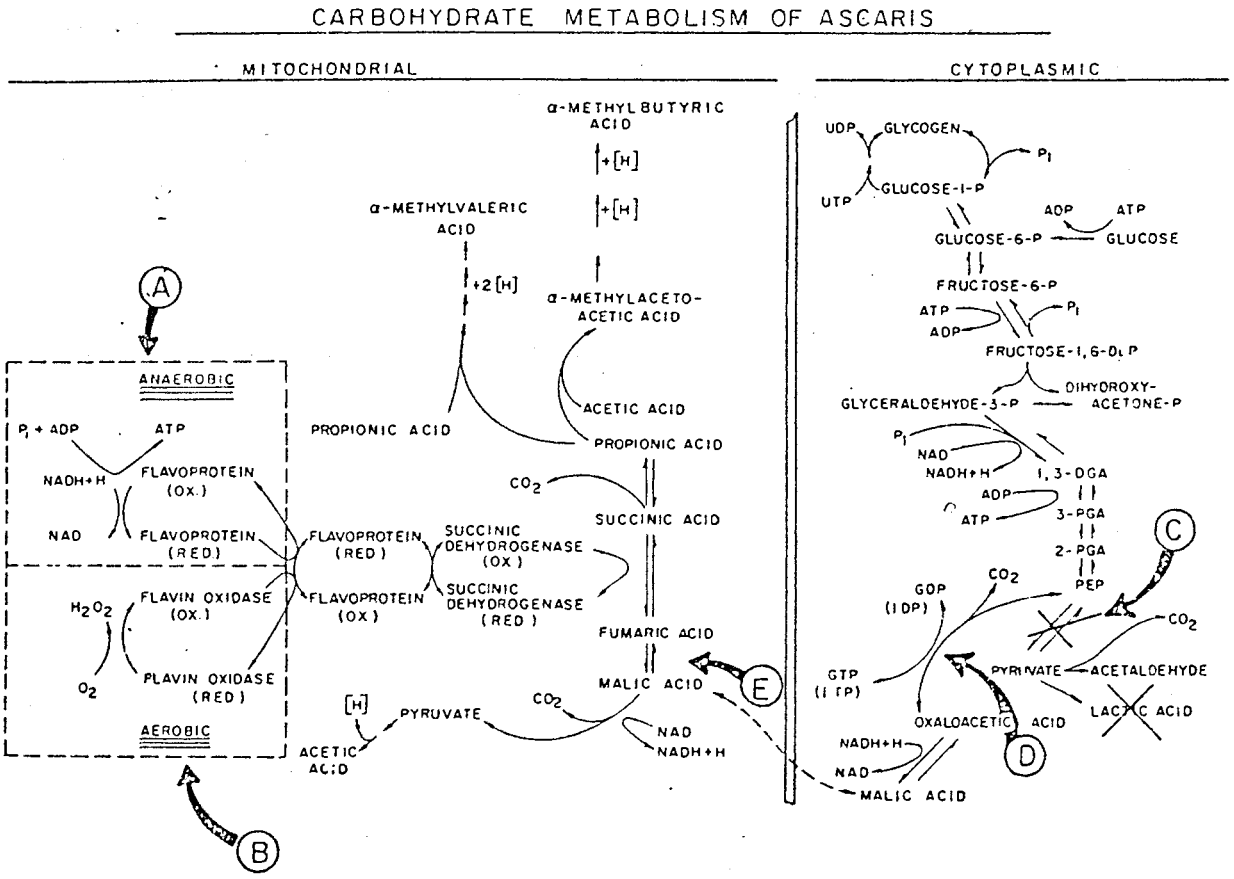


Figure 1. A Summary of Carbohydrate Metabolism for Ascaris Suum

malate which seems to be the substrate necessary for mitochondrial formation of ATP. The mitochondria of helminths do not possess oxidative phosphorylating systems, but do have a means of producing ATP from malate (Figure 1 - A). One half of the malate that enters the mitochondria undergoes oxidative decarboxylation producing pyruvate and CO_2 as well as regenerating reducing power in the form of NADH (29). The enzyme catalyzing this reaction is called the malic enzyme (29, 33). The regeneration of NADH allows for the other one half of malate to be reduced to succinate via fumarate and the enzyme, fumarate reductase (29). The formation of succinate occurs with a concomitant generation of ATP. This pathway of anaerobic metabolism is believed to be fairly common to most helminths and for this reason serves as the target for modern therapeutic agents used in the treatment of helminthiasis.

CHAPTER III

ANTHELMINTICS

The ultimate goal of pharmacological research is to produce chemotherapeutic agents that act selectively on the biochemical and physiological mechanisms essential for the survival of the invading organisms (8, 30). In the case of the helminth parasites, these chemotherapeutic agents are referred to as anthelmintics. The development of these agents has been a primary concern to many pharmaceutical researchers due to the number of men and animals with this infection. Despite the importance of a vast need for effective agents that are highly selective for the invading parasite, but with low toxicity to the host, the development of these agents has been slow. Only recently have advances in this area been made as a result of newly discovered knowledge about the biochemistry of the helminths.

With the knowledge of metabolic pathways within the parasite that differ from those of the host, the organic chemist is able to "target" the drug to control points within these helmintic pathways. However, many of the drugs used therapeutically against invading organisms offer no clue as to their exact mechanism of action. Studies, like those mentioned earlier for the determination of mechanisms of drug action, have been used by many research laboratories to explain the anthelmintic nature of various agents. For instance, in a review by Coles (6) the mechanisms of action for four anthelmintics most often used in

veterinary medicine. This review shows that Levamisole hydrochloride and Pyrantel are anthelmintics that result in the paralysis of the helminths while the class of drugs shown as the salicylanilides served to decrease the amount of ATP within the helminth with a single mode of action not likely. The fourth class of anthelmintics discussed in this review by Cole is the benzimidazoles. From the data collected by Cole, it appears that the benzimidazole compounds work by interfering with the activity of the enzyme fumarate reductase. In a later discussion of these compounds, evidence will be presented which suggests that the benzimidazole called mebendazole, also interferes with the integrity of the membranes of the intestinal epithelium.

The majority of anthelmintics used therapeutically are administered orally whether they are meant to treat parasites of the gastrointestinal tract or those of the circulatory system. In most cases, there is a rapid absorption of a portion of the drug. Very rarely is the entire amount of drug present in the stomach or intestine absorbed. Once this absorbed drug reaches the plasma it becomes equally distributed throughout all the body fluids. Distribution of this type facilitates the termination of any larval forms migrating within the body. The biotransformation of absorbed anthelmintic is generally very rapid with the metabolite having no biological activity. The liver is generally the organ of biotransformation while the kidney is the organ responsible for excreting the inactive metabolite from the body. That portion of the drug not absorbed from the gastrointestinal tract will pass out of the body as a component of the feces (11, 12, and 15).

The toxicity generally associated with anthelmintic agents is minimal. This should come as no surprise in light of the previous

discussion of "targeting" chemotherapeutic agents to metabolic systems not found in the host. Designing the drug in the form of an active substrate of helminth metabolism ensures that it will be absorbed into the parasite and regarded as foreign by the host leading to a biotransformation of the agent that enhances its excretion. Despite how this sounds, it is possible to experience abdominal distress, nausea, and diarrhea if the dose given is higher than the one suggested therapeutically.

Benzimidazoles

From the many years of research in anthelmintics came a compound that had a five-membered ring nucleus and a heterocyclic compound substituted at the number two position (4). This group of compounds had a high biological activity against invading parasites. The five-membered ring nucleus is known as the benzimidazole nucleus. These compounds are potent chemotherapeutic agents against all forms of the helminth, i.e., the larval, adult and ovicyte stages (7, 25).

The benzimidazole compounds are not generally soluble in water (23). Therefore, something must be done to compensate for this, i.e., administer in oil, alter the molecule so that solubility is increased or adjust the amount of compound given so as to assure adequate absorption. Benzimidazole compounds behave like other anthelmintics in that they are evenly distributed in the body fluids, rapidly transformed into their metabolites and excreted by the kidney (34, 35).

At the present time, there are four benzimidazole compounds of interest to pharmaceutical researchers (36). These are thiabendazole, mebendazole, cambendazole and parbendazole (Figure 2). Despite the effectiveness of these four compounds, very little is known about the

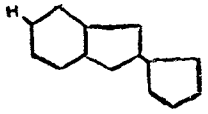
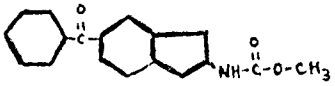
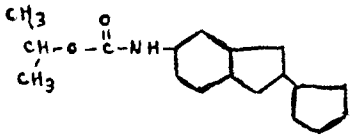
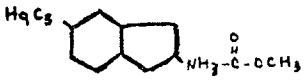
Name	Structure	Biological Activity
Thiabendazole		Gastrointestinal nematodes and eggs
Mebendazole		Gastrointestinal worms, lung worms, eggs, adult and larval cestodes
Cambendazole		Gastrointestinal nematodes, eggs, lung worm, adult cestodes
Parbendazole		Gastrointestinal nematodes eggs

Figure 2. Benzimidazole Structure and Biological Activity at Therapeutic Dosage Levels

mechanism by which these drugs act. The one benzimidazole compound that has been widely studied is mebendazole.

Methyl-5(6)-benzoyl-2-benzimidazole carbamate or mebendazole was originally studied for its effect on glucose uptake and glycogen storage within treated helminths (36). The more recent investigations of this drug, like those of Borgers et al. (3) and Van den Bossche (37), have indicated that there are ultra-structural changes occurring within the cells exposed to mebendazole. These changes have to do with the transport of secretory granules within these cells. Van den Bossche sequenced the changes he saw as the disappearance of microtubules occurring first, followed by a block in the transport of secretory granules as seen by an accumulation of these structures in the cytoplasm and finally, a necrosis of the cytoplasm (37). The disappearance of microtubules in the intestinal cells of Ascaris Suum was also observed by Borgers and his associates as seen in electromicrographs of the effected tissue. In view of this evidence, it is generally accepted that the mechanism of action for mebendazole is an interference of microtubular structures by binding to the tubulin component of these structures (6).

The similarity of cambendazole and parbendazole to mebendazole (they all have similar substituents as well as carbamate activity) should lead to the determination that these two compounds act on cells in the same fashion as mebendazole (36). Thiabendazole, on the other hand, does not exhibit any carbamate activity. Early studies involving the effects of thiabendazole on organisms seemed to indicate that the enzyme fumarate reductase was being affected. However, recent evidence indicates that the larval forms acted upon by thiabendazole do not contain the enzyme fumarate reductase (32). Since the absence of this

enzyme cannot account for the mechanism by which thiabendazole is working in this stage of helminth development, there must be other ways that this drug can affect living systems. With the amount of work being done on this drug by a number of laboratories, it should not be long before we clearly understand the mechanism of action for thiabendazole.

CHAPTER IV

SUMMARY

Pharmacology is the science that studies the interactions of chemical agents and living systems. This science has been known to man for many thousands of years. As it exists today it is a science that receives contributions from a number of different areas, including biochemistry, physiology and pathology. This cooperative nature of pharmacology has lead to an enhanced understanding of the pharmacodynamics of certain drugs.

The pharmacodynamics of a drug involve the mechanisms by which the drug is absorbed, distributed, excreted, metabolized and, most importantly, acts at the cellular level. Each of these pharmacodynamic characteristics will behave in a fashion that is determined by the chemical structure of the drug. All of these characteristics accompanying a drug must be taken into consideration when deciding how much of the drug should be given to attain therapeutic levels of the drug in the body of an individual.

Determining the exact mechanism of action associated with any drug is one of the hardest experimental problems to approach. The techniques designed for such an investigation are numerous with three such techniques being reviewed in this paper. The three techniques encompassed by this paper are enzymatic analysis, ionic flux, and micropuncture procedures.

Throughout the world there exists the problem of man and domestic animals being infected with parasitic helminths, or worms. In order that drugs can be specifically developed for the treatment of this condition, advances in the knowledge of helminth biochemistry had to be made. The knowledge gained from scientists investigating metabolism of helminths indicated that these parasites are anaerobic in nature as opposed to the aerobic nature of their hosts. Information of this kind has become a valuable tool for the organic chemists specializing in the designing of drugs specific for these anaerobes. The resulting collection of drugs are known as anthelmintics.

Chemotherapeutic agents used as anthelmintics are generally rapidly absorbed from the gastrointestinal tract, evenly distributed, quickly metabolized, and excreted in the urine. Also associated with these compounds is a low level of toxicity to the host.

A class of anthelmintics that is a major source of treating helminthiasis (the condition of having helminths) is the benzimidazoles. Included in this class of drug is thiabendazole, mebendazole, cambendazole and parbendazole. The methods of absorption, distribution, metabolism and excretion in the host are very similar to those already mentioned above. The interesting thing about the benzimidazole compounds is the mystery of how they work at the cellular level. The popular theory of how mebendazole works involves the binding of this compound to the tubulin component of microtubules which blocks microtubule formation. Due to the similarity of cambendazole and parbendazole to mebendazole, further investigations of these two compounds should indicate similar mechanisms of action with mebendazole. The amount of work that needs to be done with thiabendazole in order to

determine its mechanism of action is significant, but with the number of investigators involved, results should be obtained within the near future.

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