ABSTRACT In July 1971, the polyether ionophorous antibiotic monensin was introduced in the United States for the control of coccidiosis in poultry. At that time, prospects for new anticoccidial agents were not good. Amprolium had enjoyed several years of use, but many other compounds had been abandoned as resistance to them developed. After the introduction of monensin, most commercial broilers were medicated with the drug and it is still widely used for this purpose today. Apart from in poultry, monensin is also used to control coccidiosis in game birds, sheep, and cattle. Indeed, more animals have been medicated with ionophores, such as monensin, for control of disease than any other medicinal agents in the history of veterinary medicine. In this review, we discuss the discovery, mode of action, and efficacy of monensin, together with matters of importance to the poultry industry such as commercial use, drug resistance, toxicity, pharmacology and residues, host immunity to coccidiosis, and effects in other avian species.

Key words: monensin, ionophore, coccidiosis, poultry, *Eimeria*

INTRODUCTION

For many years, coccidiosis has been a major cause of poor performance and lost productivity in poultry and other farm animals. The disease is caused by protozoan parasites of the genus *Eimeria*, the oocysts of which can be present in the environment wherever poultry are raised. The life cycle is direct and involves oral ingestion of the infective transmission stage the sporulated oocyst, asexual and sexual reproduction (schizogony and gametogony) in the host, and completion of a new generation of oocysts about a week later (Figure 1). Each species is specific to a given host; 7 are considered to parasitize the chicken and another 7 are considered to parasitize the turkey. An important consequence of specificity is that those that parasitize poultry are not capable of establishing infection in other hosts such as cattle and sheep.

Early recommendations for controlling coccidiosis involved attention to improvements in management and hygiene. Although this is still important, the intensive nature of the poultry industry, the fact that birds are usually reared in direct contact with their feces, and the resilience of oocysts ensures the continued presence of coccidia wherever poultry are raised. Attempts to eradicate the disease have been unsuccessful. This situation was transformed in the 1940s by the introduction of sulfamethazine and recognition that this compound could be incorporated in feed during the rearing phase of poultry production, by which means infection could be prevented (Campbell, 2008; Chapman, 2009). Prevention (prophylaxis) has been the mainstay of poultry-meat production ever since. Sulfamethazine was succeeded by many other drugs synthesized in pharmaceutical laboratories (synthetic drugs or so-called chemicals). The Achilles’ heel of chemotherapy has always been the acquisition of drug resistance by parasites. First reported in the 1950s, by the 1970s, resistance had become widespread and was reported throughout the world where poultry production was a major industry.

In 1967, the structure of monensic acid (subsequently known as monensin) was described and the compound was reported to have broad-spectrum anticoccidial activity (Agtarap et al., 1967). This discovery led to the introduction of further compounds with a similar mode of action. Monensin is an antibiotic produced as a byproduct of fermentation by *Streptomyces cinnamonensis* and belongs to a family of drugs known as polyether antibiotics or ionophores. Although some anticoccidial activity has been demonstrated in other antibiotics (e.g., tetracyclines, lincomycin, and spiramycin), monensin was the first to show an effect at practical concentrations for incorporation in feed (Ryley and Betts, 1973).

Unlike antibiotics that are used by the poultry industry for growth enhancement, monensin is unusual
because it has a mode of action that specifically targets the *Eimeria* parasite. Many aspects of the use of monensin have been controversial, but its success in controlling coccidiosis in poultry is undeniable. Today, alternatives to medication for this disease are actively sought, but for the last 40 yr, control has depended largely upon this and other ionophores. Several reasons may explain the continued use of monensin. First, the development of resistance has proved to be very slow (Chapman, 1984a). Second, monensin prevents the clinical effects of coccidiosis while allowing birds to acquire natural immunity to *Eimeria* infections (Chapman, 1999).

An enormous body of literature is available for monensin (a quick search of Science Direct lists 6,233 publications, of which 918 are concerned with chickens). In this review, we discuss selected aspects such as discovery, mode of action, efficacy, and practical use of monensin as well as matters of importance to the poultry industry such as toxicity, pharmacology, drug residues, drug resistance of coccidia, and acquisition of immunity by poultry to coccidiosis. Our review is focused principally on the use of monensin as an anticoccidial in broiler production. There are many references to the use of monensin in the turkey; these are documented in a recent review (Chapman, 2008).

**DISCOVERY**

The early history of the discovery of polyether antibiotics has been reviewed by Westley (1982). Nigericin and compound X-537A (later named lasalocid A) were described in 1951 (Berger et al., 1951; Harned et al., 1951). These compounds showed activity against gram-positive bacteria and mycobacteria but had no effect against gram-negative bacteria, or unspecified protozoa. At the time, they were not known to be polyether antibiotics and were rather toxic to mice. Sixteen years later, in a joint publication from the Lilly Research Laboratories, and the Department of Biochemistry of Indiana University School of Medicine, the chemical structure of a biologically active compound (monensic acid) produced by a new strain of *S. cinnamonensis* was reported (Agtarap et al., 1967). This was the first detailed description of the structure of a polyether antibiotic. At the Seventh Interscience Conference on Antimicrobial Agents and Chemotherapy in 1967 held in Chicago, 6 papers were presented by Eli Lilly and Company describing the discovery and isolation of monensin and its fermentation properties, chemistry, related compounds, anticoccidial activity, and assay (Agtarap et al., 1967; Donoho and Kline, 1967; Gorman et al., 1967; Haney and Hoehn, 1967; Shumard and Callender, 1967; Stark et al., 1967). The Institute for Enzyme Research, University of Wisconsin-Madison, provided insight into the mode of action (Estrada et al., 1968).

The events that led to the discovery and early development of monensin are remembered by one of the scientists involved, Ray Shumard (personal communication). In the 1940s through the 1960s, Eli Lilly and Company had an extensive program looking for new antibiotics produced from soil samples collected throughout the world. *Streptomyces cinnamonensis* was obtained from such a sample collected in Arizona in 1960. Mycelia from a crude fermentation broth were examined in a variety of primary biological screens, revealing weak antibiotic activity. In 1962, chicks were fed a ration containing the mycelia and marginal anticoccidial activity was demonstrated against the Wisconsin strain of *Eimeria tenella*. A development team was formed from several divisions of the corporation to isolate the active ingredient and then to undertake the monumental task of taking this discovery forward for US Food and Drug Administration approval, a process that consumed millions of dollars in research, development, and capital investment in fermentation facilities. The project had many fits and starts and was halted on more than one occasion, primarily due to concerns about production costs and eventual profitability of the venture. The decision to develop monensin was vindicated after marketing in 1971 when its benefits in controlling coccidiosis were perceived by the broiler industry; indeed, the product was so successful that initially fermentation facilities could not keep up with demand. A major production improvement came when US Food and Drug Administration approval was obtained for use of monensin containing mycelia as the product rather than the more expensive crystalline form of the drug.
STRUCTURE AND ION TRANSPORT

The term polyether antibiotic refers to complex molecules with antimicrobial activity comprising many cyclic ether groups (Westley, 1982). In the case of monensin, the molecule is pentacyclic with side chains comprising 7 methyl, an ethyl, and carboxyl and hydroxyl groups (Aggarap and Chamberlin, 1967). The molecular formula is C_{36}H_{62}O_{11} and the structure is shown in Figure 2A. The biosynthesis of monensin by *S. cinnamonensis* has been studied and the molecule is shown to be synthesized from 5 acetate, 7 propionate, and 1 butyrate components (Day et al., 1973). The 3-dimensional conformation of monensin and other ionophores is still under investigation (Przybylski et al., 2007).

Monensin can assume a cyclic conformation in which the oxygen atoms are at the center of a doughnut-shaped structure in which they can complex with suitable cations (Figure 2B). The alkyl groups are spread out over the outer surface and render the complex highly soluble in the lipid component of cell membranes. These properties allow ionophores to enter cell membranes and conduct cations across them down their concentration gradients by the process of passive diffusion (Pressman, 1968). According to Pressman (1976), his laboratory chose the term ionophore, meaning ion bearer, to indicate this dynamic ion transport mechanism. Monensin belongs to a class known as carboxylic ionophores that have an open chain containing oxygenated heterocyclic rings and a single terminal carboxyl group. The ring conformation is formed by head-to-tail hydrogen bonds. In the case of monensin, the carboxyl group is not involved in ligand formation with cations.

Ionophores differ in their cation selectivity, monensin showing preference for Na\(^{+}\) ions followed by K\(^{+}\), Rb\(^{+}\), and Li\(^{+}\) thus forming complexes with monovalent ions. Only lasalocid, another ionophore, can complex with divalent ions, such as Ca\(^{2+}\), with the same affinity (Westley, 1982). The mechanism of ion transport involves diffusion of the protonated ionophore through the cell membrane and release of H\(^{+}\) ions at the polar interface with the cytoplasm in exchange for cations (Pressman, 1976). The ionophore then diffuses to the opposite surface where the cations are released; acquisition of further H\(^{+}\) ions allows this process to be repeated. Carboxylic ionophores are exchange diffusion carriers and allow the diffusion of ions down concentration gradients that occur in the cell. They have proved useful tools in investigating ion transport across cell membranes and have an extremely diverse range of effects in many cell types and tissues (Reed, 1982).

MODE OF ACTION

Several theories have been put forward to explain the mode of action of ionophores against coccidia (Chapman, 1984a). Monensin inhibits the transport of K\(^{+}\) ions into mitochondria obtained from rat liver, resulting in the inhibition of substrate oxidation and adenosine triphosphate hydrolysis (Estrada et al., 1968). It was suggested, therefore, albeit with no evidence, that the drug may have a similar effect upon the mitochondria of coccidia (Ryley and Betts, 1973). Monensin causes vacuolation and swelling of intracellular sporozoites of *E. tenella* at concentrations that have no apparent effect upon the parasite mitochondrion; it is unlikely, therefore, that this organelle is directly involved in the mode of action of ionophores (Smith and Strout, 1979, 1980a). Electron micrographs of an untreated sporozoite of *E. tenella* and a sporozoite treated with monensin showing the large vacuole and obvious swelling are presented in Figure 3A and B.

An alternative hypothesis is that abolishment of cation gradients across the host cell membrane by ionophores inhibits active transport of carbohydrate and deprives the developing parasite of nutrients (Wang, 1978, 1982). However, the host cell appears to be unaffected by concentrations of drug that are lethal to intracellular sporozoites; once intracellular, the parasite is no longer susceptible to the action of the drug (Smith and Strout, 1980a).

Sporozoites are able to invade cultured cells when they are grown in appropriate media and this system has been useful in investigations of the mode of action. It has been shown that ionophores are accumulated by sporozoites of *E. tenella* and that the coccidioidal ef-
fect may be expressed before cell penetration (Itagaki et al., 1974; Smith and Strout, 1979). Inclusion of monensin in culture medium also results in degeneration of sporozoites after they have invaded host cells and subsequent development is inhibited (Strout and Ouellette, 1973; McDougald and Galloway, 1976). These studies were carried out with the drug continuously present in the culture medium and therefore provided no information on the role of the host cell. However, Smith et al. (1981) showed that if sporozoites of *E. tenella* are pretreated with ionophores and the drug is then removed by thorough washing, significant inhibition occurs both of host cell penetration and subsequent asexual development. This suggests that the host cell is not necessary for the expression of anticoccidial activity and that, for in vivo activity, sporozoites must acquire the drug before invasion.

Monensin causes an increase in Na\(^+\) ion influx and stimulation of \((\text{Na}^+\text{-K}^+)\)-ATPase that pumps excess Na\(^+\) ions out of the sporozoite (Smith and Galloway, 1983). This process requires energy and results in enhanced utilization of adenosine triphosphate. Lactate production is increased, which indicates a stimulation of glycolysis, and there is a concomitant increase in the utilization of amylopectin. It is suggested that accumulation of Na\(^+\) ions causes water to enter by osmosis and the parasite swells and eventually bursts. The site of lethal activity of ionophores was considered the outer membrane (the pellicle) of the sporozoite. Differential activity between host cell and parasite might be due to differing chemical compositions of their respective membranes such as a difference in aqueous-hydrocarbon distribution (Smith and Strout, 1979).

Another more recent explanation of the mode of action of monensin is that the drug is able to interrupt host cell invasion by sporozoites (del Cacho et al., 2007). The outer membrane of the sporozoite contains lipid rafts that are involved in many biochemical processes such as signal transduction, membrane trafficking, and molecular sorting. A resident protein of lipid rafts, flotillin-1, was identified in sporozoites of *E. tenella* at the apex of the cell, a region that mediates cell invasion. Monensin was found to disrupt the localization of flotillin-1 within raft structures, resulting in the loss of ability to invade host cells. This effect was significantly reduced in a monensin-resistant line of *E. tenella*.

In addition to an effect against sporozoites, monensin is effective against merozoites, presumably by the same mechanism. Merozoites are the motile forms that are released into the intestinal lumen after schizogony, later during the life cycle (Long and Jeffers, 1982). A coccidioidal effect of monensin against merozoites was also reported by Mehlhorn et al. (1983), but in their studies, no effect was seen against sporozoites, for which no explanation was provided. Monensin has no effect upon intracellular macrogametes, the sexual stage of the life cycle (Long and Jeffers, 1982). Whether it affects microgametes during the brief period they spend in the intestinal lumen is unknown.

It is surprising that motile stages remain extracellular for sufficient time to accumulate a lethal quantity of drug, but the uptake of monensin by sporozoites is extremely rapid (C. K. Smith II, personal communication). The action of monensin in vivo has been demonstrated by harvesting sporozoites from the intestinal contents of medicated birds, shortly after inoculation of oocysts, and inoculating them into chicken embryos when they were not as infective as sporozoites obtained from unmedicated chickens (Long et al., 1983).

It has been suggested that monensin may reach the parasite via a systemic route after absorption rather than by direct contact in the intestinal lumen because lesions due to *E. tenella* were reduced in medicated birds if the ceca (where this species develops) are ligated (McQuistion and McDougald, 1979). Long and Jeffers (1982), however, suggested that ligation may not prevent the drug from passing through the neck of the cecum. In view of the extremely low concentration of monensin present in serum, C. K. Smith II (personal communication) believes it is highly improbable that

![Figure 3. A) Normal sporozoite of *Eimeria tenella*. B) Sporozoite of *E. tenella* treated with 1.0 µg/mL of monensin. Note vacuolation and typical ballooning (Smith et al., 1981). Reproduced with permission from C. K. Smith II and the *Journal of Parasitology*, Allen Press Publishing Services.](image-url)
an effective level of monensin could reach intracellular parasites by a systemic route.

**Efficacy**

The introduction of monensin was accompanied by the commercial release of extensive data concerning efficacy in battery, floor-pen, and field studies on a scale hitherto unknown for any previous drug (Anonymous, 1974). A selection of the relevant literature is reviewed here (for early references, see Ruff, 1982). Initial experiments involved cage studies in which the effects of medication were determined during the acute phase of infection. In the first such study, 121 mg/kg of monensin in the feed was shown to be effective against all species then recognized in the chicken (Shumard and Callender, 1967). Eight isolates of *E. tenella*, 4 each of *Eimeria necatrix* and *Eimeria acervulina* and 2 each of *Eimeria brunetti*, *Eimeria maxima*, and *Eimeria mitvati*, were included in this investigation. Several criteria were employed to determine efficacy, including mortality, weight gain, intestinal lesions, and oocyst production. Although some lesions and oocysts were produced in medicated birds, it was concluded that monensin had a broad-spectrum effect against *Eimeria* species of the fowl. Subsequently, efficacy has been demonstrated against other species that infect poultry (Watkins et al., 1990).

In a study conducted in the United Kingdom, complete control was not achieved in battery experiments irrespective of the criterion used to assess infection and it was concluded that monensin "is not very impressive by comparison with the other drugs in stringent laboratory tests" (Ryley and Wilson, 1975). This led to considerable controversy concerning the validity of the screening methods used to discover anticoccidial activity. Several aspects of experimental design may have contributed to the difficulty of demonstrating monensin efficacy. First, a wheat-based diet, rather than a maize-based diet as used in the United States, was employed and it was subsequently shown that monensin is less effective in the wheat-based diets used in the United Kingdom (Williams, 1992). Second, medication was initiated 24 h before infection, but better efficacy was achieved if medication began 48 h before infection, indicating that the drug may require time to achieve an optimum concentration in the gut. Third, massive doses of oocysts were administered to the birds (403, 257, 250, and 4,000 × 10^3 of *E. tenella*, *E. necatrix*, *E. brunetti*, and *E. acervulina*, respectively) and this may have overwhelmed the effect of the drug (Callender, 1978). Finally, the diet used was intentionally deficient in vitamin K to achieve high mortality due to hemorrhage in unmedicated controls infected with *E. tenella* or *E. necatrix*.

The efficacy of monensin has been confirmed in numerous floor-pen experiments, which are not reviewed here. The drug is often used as a reference anticoccidial in such experiments for comparison with other anticoccidial agents (Shumard and Callender, 1967; Shumard et al., 1970; Reid et al., 1972, 1975; Clarke et al., 1974; Ruff et al., 1976; Danforth et al., 1977; McDougald et al., 1990, 1996; Conway et al., 2001). Monensin has also been used for comparisons with anticoccidial vaccines in field trials (Williams and Gobbi, 2002).

Under field conditions, monensin proved to be extremely effective and within a short period after its introduction, most commercial broilers were reared with the drug in the feed. Few published reports document the efficacy of monensin (or other drugs) in large-scale comparative commercial trials, possibly because of the difficulties in undertaking such studies. MacPherson (1978) showed that at 3 farms, broilers given monensin had improved live weights and reduced feed conversion ratios, mortality, and lesions compared with those given an amprolium combination. Jeffers et al. (1988) found that in 9 field trials, birds receiving monensin (100 or 121 mg/kg) had lower BW than birds given narasin (80 mg/kg), but feed conversions were similar with both drugs. In commercial broilers, a change to monensin from an amprolium combination resulted in an improvement in feed conversion and was accompanied by a marked reduction in the level of infection, judged by the numbers of oocysts in the litter, an effect still evident after 11 flocks (Long et al., 1975).

**Commercial Use**

In the United States, monensin is approved at a use level of 99 to 121 mg/kg in the chicken as an aid in the prevention of coccidiosis caused by 6 species of *Eimeria* (Anonymous, 2009). Monensin may be included in different feeds as the sole drug during the life of a flock (single-drug program) or in so-called shuttle programs in which monensin and another drug (ionophore or synthetic compound) are used in different feeds in a single flock. Studies conducted in the 1970s, in which birds were given monensin alone or in shuttle programs with various synthetic drugs, gave equivocal results (Kohls, 1974; Gard et al., 1978). There is little recent published information comparing the merits of using monensin in different feeds provided to broiler chickens. In one study, BW and feed conversion of birds given nicarbazin in the starter feed and an ionophore in the grower feed were similar to those of birds given ionophores in both feeds (Watkins and Bafundo, 1993).

A database is available that provides monthly information on the use of drugs by broiler-rearing complexes throughout the country (Agri Stats Inc., Fort Wayne, IN). Information is provided for 4 successive feeds referred to as starter, grower, first withdrawal, and final withdrawal feeds, respectively. Analysis for March during 2000 to 2009 indicates that the principal use of monensin is in shuttle programs involving a synthetic drug such as nicarbazin or another ionophore in the starter feed and monensin in the grower feed (28.3% of complexes). It was used in both starter and grower feeds (single-drug programs) by 8.9% of complexes.
Monensin is principally employed in grower feed, occasionally in the starter or first withdrawal feed, but not in the final withdrawal feed. Data from the United States for 2009 indicate that few complexes use monensin at its maximum permitted concentration of 121 mg/kg; 76.3% of complexes employed 110 mg/kg and 21.6% of complexes employed 99 mg/kg of the drug. Analysis for 2000 to 2009 is presented in Figure 4. A seasonal difference is evident, monensin being used primarily in winter and spring (December through May) with a decline through summer and fall (June through November). The principal reason for this is the belief that monensin depresses feed intake during warmer months of the year (see later). Widespread practice, therefore, involves the use of rotation programs in which monensin is changed to other ionophores in summer and fall.

In the European Union (EU), approximately 84% of broiler starter, grower, and finisher feeds contain an anticoccidial drug. Data for western Europe indicate that the number of birds fed monensin increases from September, peaking in December and January, and subsequently declines during the spring and summer months (C. Bostvirenois, Elanco Products Inc., personal communication). The explanation for this pattern is similar to that in the United States in that producers (especially in southern Europe) switch to other drugs believed not have an anorexic effect during hot weather. In North Africa and Middle Asia, seasonal variation in monensin use is also evident. Thus, during the grower phase of production in 2006 to 2009, the mean number of birds fed monensin in successive quarters (January to March, April to June, July to September, and October to December) was 80.4, 64.2, 45.6, and 81.8 million birds, respectively, indicating a decrease in usage during the summer months (K. L. Watkins, Elanco Products Inc., personal communication). During this period, the use of other drugs, such as narasin, increased. Monensin is also employed in other large broiler-producing regions, such as Central and Latin America and the Far East, but the extent of such use is not documented.

Monensin has been employed during the rearing phase of broiler breeder and replacement layer production. In broiler breeder pullets fed ad libitum, monensin at a concentration of 110 mg/kg interfered with the development of immunity to *Eimeria* species but did not do so when birds were given a restricted feed intake regimen (Ruff and Chute, 1980). Suboptimal concentrations are often used to permit the development of immunity (Long et al., 1979). In the United States, monensin is not approved for use in laying chickens or birds more than 16 wk of age. Monensin reduced fertility when given at 100 mg/kg to broiler breeders but had no effect on egg production, egg weight, or egg hatch (Jones et al., 1990).

**IMMUNITY**

Exposure to infection with *Eimeria* species can result in the development of immunity and protect birds against coccidiosis. Few drugs, including monensin, are capable of preventing some degree of infection; therefore, acquisition of immunity is a real possibility providing sufficient parasites are present in the environment (Chapman, 1999). After the introduction of monensin, it was considered that the drug prevented the acquisition of immunity (Reid, 1972), but several studies have shown that this is not necessarily the case. Thus, immunity developed to *E. acervulina*, *E. brunetti*, *E. maxima*, *E. mivati*, and *E. tenella* but not *E. necatrix* in chickens reared in floor pens and medicated with 121 mg/kg of monensin (Reid et al., 1972). Similar results were obtained by Callender and Shumard (1973), although they found that birds medicated with monensin remained susceptible to *E. tenella* and *E. necatrix*. In subsequent studies, immunity to various species of *Eimeria* was not prevented by monensin in some experiments but was strongly suppressed in others (Reid et al., 1977; Karlsson and Reid, 1978). Not surprisingly, the degree of immunity was greater in birds given lower concentrations of the drug, a conclusion supported by the findings of Long et al. (1979).

Experiments were carried out in battery cages in which chickens fed monensin continuously were infected with 13 repeated inocula of 1,000, 100, or 10 oocysts of *E. maxima*, *E. brunetti*, or *E. tenella* (Chapman, 1978). Results are summarized in Table 1. Birds were challenged after cessation of oocyst production and it was found that immunity had developed after repeated inoculation with 100 oocysts of *E. maxima* or 1,000 oocysts of *E. brunetti*. Partial immunity developed in chicks infected with 100 oocysts of *E. brunetti* or *E. tenella*. Thus, a protective immune response can result if medicated birds are repeatedly exposed to small numbers of oocysts. There is a direct relationship between the level of infection and degree of protection achieved,
and the level of immunity will vary depending upon the species of *Eimeria* involved.

In some experiments reported, the strains of coccidia used were obtained from birds that had not previously been exposed to monensin. Because many field strains currently show reduced sensitivity, it is now less likely that monensin will interfere with the development of immunity. Indeed, Jeffers (1989) believed that immunity may account for the effectiveness of ionophores in the field. Under practical conditions, the development of immunity will depend upon the frequency and magnitude of exposure to infection, the concentration of drug in the feed, the species of *Eimeria* involved, and their degree of drug resistance.

**SELECTION OF RESISTANCE**

Initial attempts to induce resistance to monensin in chickens were unsuccessful (Shumard et al., 1970; Chapman, 1976; Weppelman et al., 1977a; Ruff et al., 1985). Chapman (1984b) propagated the drug-sensitive Houghton strain of *E. tenella* in groups of 30 birds each infected with $10^6$ oocysts and medicated with 100 mg/kg of monensin, and after 16 passages, a partially resistant line was produced. Passage of this line in birds medicated with 200 or 300 mg/kg of monensin did not increase the degree of resistance despite the increase in drug selection pressure. Five passages in nonmedicated chickens resulted in restoration of sensitivity, suggesting that resistance was unstable or that drug-sensitive parasites had not been eliminated and in the absence of drug selection pressure. Five passages in nonmedicated chickens resulted in restoration of sensitivity, suggesting that resistance was unstable or that drug-sensitive parasites had not been eliminated and in the absence of selection pressure came to dominate the parasite population. In a subsequent study, however, resistance to monensin was not lost in field isolates of *E. tenella* after 10 passages in unmedicated birds (Chapman, 1986a). A 2-fold increase in resistance to monensin was induced in the drug-sensitive Weybridge strain of *E. tenella* after a single passage in birds medicated with 250 mg/kg of the drug (R. B. Williams, unpublished data). More recently, using the Houghton strain of *E. tenella*, resistance was induced after 35 passages in chickens medicated with 100 to 200 mg/kg of monensin (Wang et al., 2006).

The most extensive investigation involved propagation of *E. acervulina* for 60 generations in medicated birds (Bafundo and Jeffers, 1990). A decrease in sensitivity to monensin and a mixture of monensin and nicarbazin, measured by an increase in parasite reproductive capacity, was noted. Resistance was also demonstrated after propagation of a field strain of *E. tenella* (obtained from a broiler flock in 1991) in chickens medicated with 200 mg/kg of monensin (Zhu and McDougald, 1992). However, interpretation of that result is difficult because it was not stated whether the strain was drug-sensitive at the outset of the experiments. Resistance may be amplified by serially treating sporozoites from a monensin-resistant field strain of *E. tenella* with monensin in vitro (Zhu et al., 1994a).

**RESISTANCE IN THE FIELD**

It is generally accepted that resistance has been slow to develop in the field and that this partly explains the longevity of monensin compared with earlier synthetic drugs. According to Smith and Galloway (1983), the action of monensin against sporozoites is directly related to a general biochemically nonspecific effect involving transmembrane sodium transport and this may explain why resistance has been slow to occur. Alternatively, the selection pressure imposed when birds are fed normal use levels may be insufficient to select for resistance and eliminate drug-sensitive parasites.

The most extensive series of investigations into the occurrence of drug resistance in the field were conducted by T. K. Jeffers in the early 1970s. Resistance to monensin was first documented in isolates obtained from broiler farms in the United States during a 48-mo period from 1970 to 1973 (Jeffers, 1974a). Two isolates of *E. maxima* were resistant out of 30 examined but none were resistant of the 165 *E. acervulina* or the 61 *E. tenella* isolates tested (Jeffers, 1974a,b). Further isolates of *E. acervulina*, *E. maxima*, and *E. tenella* were collected during 1975 to 1977, none being resistant to monensin (Jeffers, 1978a,b).

In the United Kingdom, isolates of *E. maxima* from sites where monensin had been used for 11 successive crops produced more oocysts in medicated birds than isolates from sites where monensin had not been employed, but no differences were apparent when weight gain was used to measure infection (Chapman, 1979). In a later study, monensin was shown to be less effective, judged by weight gain and oocyst production, against isolates of *E. acervulina* from broiler flocks reared where the drug had been used extensively than

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**Table 1. Immunity development in chickens medicated with monensin (125 mg/kg) and given 13 doses of 3 species of *Eimeria*¹**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Number of oocysts given (13 times)</th>
<th><em>Eimeria maxima</em></th>
<th><em>Eimeria brunetti</em></th>
<th><em>Eimeria tenella</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Monensin</td>
<td>10</td>
<td>Partial</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Monensin</td>
<td>100</td>
<td>Yes</td>
<td>Partial</td>
<td>Partial</td>
</tr>
<tr>
<td>Monensin</td>
<td>1,000</td>
<td>Yes</td>
<td>Yes</td>
<td>Partial</td>
</tr>
<tr>
<td>None</td>
<td>10</td>
<td>Yes</td>
<td>Yes</td>
<td>Partial</td>
</tr>
</tbody>
</table>

¹Data from Chapman (1978).

²Based upon weight gain after challenge in comparison with challenged and unchallenged controls.
against broiler breeder isolates from flocks reared where the drug had never been employed (Chapman, 1982). A similar study conducted in the United States showed that about half of the isolates examined from broiler farms showed reduced sensitivity to monensin, but almost all from broiler breeder farms were sensitive (Mathis et al., 1984). In another investigation, all strains tested showed some response to monensin (McDougald, 1981). More recent studies indicate a continued decline in ionophore efficacy. For example, Mathis (1999) found that monensin and other ionophores were only marginally or poorly effective; similar conclusions have been reached by others (Chapman and Hacker, 1994). There have been many reports (not reviewed) from different countries of resistance to monensin.

In view of reports of resistance to ionophores, it is surprising that outbreaks of coccidiosis do not occur more frequently in flocks given these drugs. Some workers have questioned whether the methods used to determine resistance are appropriate (Watkins, 1997). Conventional methods involve challenging birds with a dose of oocysts large enough to cause a depression in weight gain or lesions in the intestines, or both. Such doses may be much greater than those likely to be encountered in the field. However, experiments with isolates considered resistant showed lack of control irrespective of whether a large or small dose of oocysts was administered (Chapman and Shirley, 1989).

An explanation for the apparent efficacy of monensin under commercial conditions must be sought elsewhere. Despite the presence of resistant strains, it is likely that at well-managed sites, the numbers of oocysts are insufficient to cause clinical disease. Rotibi et al. (1989) have pointed out that drug-sensitivity tests may give an indication of the potential for outbreaks of coccidiosis during periods of high infection pressure. According to Jeffers (1989), in most studies, a complete loss of drug efficacy has not been reported. It is likely that partial loss of efficacy combined with the acquisition of immunity explains the continued efficacy of monensin in the field.

**MECHANISM OF RESISTANCE**

Augustine et al. (1987) showed that the uptake of monensin by sporozoites of *E. tenella* resistant to the drug was significantly less than that by sensitive sporozoites. The amount of drug required to inhibit development was 20 to 40 times higher for resistant parasites than for sensitive parasites. It was concluded that differences in ionophore accumulation may reflect the degree of resistance. Fundamental changes in biophysical properties of the parasite cell wall may be required to cope with a nonspecific action upon transmembrane cation transport (Jeffers, 1989). This might not be rapidly accomplished even with intense selection pressure for resistance.

Two proteins with molecular weights of approximately 50.0 and 31.4 kDa were identified in sporozoites of monensin-resistant lines of *E. tenella* but not in sporozoites of a sensitive parent line (Zhu et al., 1994b). Whether they are associated in some way with drug resistance is not known.

In experiments with the microorganism *Acholeplasma laidlawii*, the efficacy of narasin was influenced by the composition of the cell membrane, a greater proportion of unsaturated lipids resulting in increased activity (Smith and Strout, 1980b). The authors reasoned that greater unsaturation of membrane lipids would result in increased membrane fluidity and this could enhance the ability of the drug to act as a mobile carrier of cations. It has also been suggested, albeit with no evidence, that resistance to ionophores may also involve changes in membrane permeability of *Eimeria* (Chapman, 1997). Recent evidence to support this was obtained by Wang et al. (2006), who found that membrane fluidity of monensin-resistant lines of *E. tenella* was lower than that of a sensitive line. However, no description of the method by which membrane fluidity was determined was provided.

Analysis of differentially expressed genes by a monensin-resistant line of *E. tenella*, using cDNA array, indicated a 6-fold upregulation of genes mainly involved in cytoskeletal rearrangement and energy metabolism compared with a sensitive parental line (Chen et al., 2008). The relevance of these observations to mechanisms of resistance is unclear.

**CROSS-RESISTANCE**

Drugs with a similar mode of action, such as ionophores, are likely to share a similar mechanism of resistance. This phenomenon is known as cross-resistance and should be distinguished from multiple resistance, which results from separate exposure to the drugs in question; both have been described in the case of ionophores. Several studies have reported cross-resistance between monensin and other ionophores (Ryley, 1980; Jeffers, 1984; Stallbaumer and Daisy, 1988). The situation regarding lasalocid and maduramicin is unclear. Isolates of *E. tenella* from sites where monensin had been used, but not lasalocid, were resistant to both drugs (Chapman and Shirley, 1989), indicating that resistance is shared (cross-resistance). In another study, however, fewer oocysts were produced by birds given lasalocid than birds given monensin, salinomycin, or narasin (Chapman, 1986b). Nevertheless, substantial numbers of oocysts were produced with all 4 drugs, suggesting that resistance is shared. Other studies have suggested that lasalocid is able to control strains resistant to monensin (Weppelman et al., 1977b; Bedrník et al., 1989). It has been suggested that the mode of action of lasalocid, which is capable of transporting divalent as well as monovalent ions, may be somewhat different from that of monensin, although no supporting evidence has been provided (Weppelman et al., 1977b). There have been fewer studies with maduramicin. A high degree of cross-resistance was reported by Raether...
and Paefgen (1989), but McDougald et al. (1987) and Bedrník et al. (1989) found that maduramicin was effective against strains resistant to monensin and other ionophores. In practice, the widespread use of ionophores has resulted in extensive multiple resistance to these drugs. No cross-resistance was found between monensin and 13 synthetic drugs to which strains of E. tenella were resistant (McLoughlin and Chute, 1974).

**RESTORATION OF SENSITIVITY**

Restoration of drug sensitivity may occur after change from one anticoccidial drug to another with a different mode of action or by relaxation of selection pressure, and this provides a justification for alternation of drugs in coccidiosis control programs. Thus, the sensitivity of E. acervulina to arprinocid was partially restored after propagation of a resistant strain in birds given monensin or no medication (Tamas and Olson, 1986). Similarly, the sensitivity of E. tenella to amprolium was restored after use of monensin (Ruff and Chute, 1985). There seem to have been no investigations into the restoration of sensitivity to monensin by the use of other drugs.

Restoration of sensitivity may result from the introduction of drug-sensitive parasites that will outnumber and interbreed with resistant parasites, thus reducing the level of drug resistance of the coccidial population. In the United States, partial restoration of sensitivity to monensin was demonstrated after the use of an anticoccidial vaccine comprising drug-sensitive strains that were isolated before monensin was introduced (Chapman, 1994). This observation formed the basis of a practical recommendation for rotation programs in which the use of monensin in broilers is alternated with the use of such vaccines (Chapman et al., 2002). Improved efficacy of monensin after use of a drug-sensitive vaccine was also found in a study conducted in the EU (Peek and Landman, 2006).

A proposal for a yearly rotation program in which 6 successive broiler flocks receive medication or are vaccinated is shown in Figure 5. It is suggested that medication involves shuttle programs in which synthetic drugs are used in the starter feed followed by monovalent and divalent ionophores such as monensin and lasalocid in the grower feed. Many other drug permutations are possible. In the case of synthetic drugs, compounds known to have a different mode of action should be used. The use of monovalent and divalent ion-carrying ionophores assumes that reported differences in the mode of action between these types of ionophores are correct. It should be noted that appropriate choice of drugs will be better facilitated if the drug sensitivity of strains of Eimeria at the farms involved is known; however, this is rarely the case. It is proposed that birds be vaccinated for the flock after clean-out of old litter because this should help reduce numbers of any resistant parasites present in the environment of the poultry house. A vaccine should be used that comprises live oocysts of strains of Eimeria known to be drug-sensitive to introduce to the house organisms that subsequently will increase sensitivity of the resident coccidial population to anticoccidial drugs. The proposal presented in Figure 5 is tailored to existing practices in the United States where it is customary to use vaccines in the summer months and shuttle programs at other times of year.

**DRUG COMBINATIONS**

Combinations of monensin and clopidol (McDougald, 1977), monensin and lasalocid (McDougald, 1978), and monensin and nicarbazin (Callender and Jeffers, 1980) are said to be synergistic; that is, their combined potency is greater than the sum of the potencies of the separate ingredients. However, no combinations of monensin with other anticoccidial drugs are approved for commercial use. Monensin is often used in broiler feeds supplemented separately with roxarsone, an arsenical growth enhancer. Mixtures of these drugs have been shown to give improved control of isolates that are resistant to ionophores (McDougald et al., 1992).

**DIETARY INTERACTIONS**

In 1978, a commercial broiler production facility was leased by Lilly Research Laboratories in Alabama and subsequently in Arkansas to pursue monensin-nutrition interactions under field conditions (R. H. Wellenreiter, Lilly Research Laboratories, unpublished data). Mon-
riboflavin in maize than in wheat. Vitamin A and E concentrations (Parsons and Baker, 1982; Welch et al., 1988). Monensin is less effective in chickens fed a wheat-based diet rather than a maize-based diet (Williams, 1992). This was attributed to the reduced pathogenicity of E. tenella in maize-fed birds, possibly due to the protective effects of the higher concentrations of vitamins A and E and lower levels of niacin and riboflavin in maize than in wheat.

**PHARMACOLOGY AND ENVIRONMENTAL FATE**

The pharmacology and toxicology of monovalent carboxylic ionophores have been reviewed by Pressman and Fahim (1982). Monensin when administered orally is absorbed, metabolized, excreted in bile, and eliminated in the feces. A bioautographic procedure, after medication with 121 mg/kg of monensin in the feed of chickens, indicated that at zero time withdrawal fat contained approximately 0.1 mg/kg, whereas no detectable residues were present in liver, kidney, or muscle (Donoho and Kline, 1967). Studies with a radiolabeled drug showed that after 3 days withdrawal from feed, all edible tissues except liver were at or below 0.05 mg/kg of total radioactivity (Donoho et al., 1982). In other studies, however, in which a sodium partitioning method was employed, concentrations in tissue were an order of magnitude higher than bioautography values (Pressman and Fahim, 1982). It was proposed that the latter method was subject to nonspecific interference and gave results unrelated to monensin concentrations in the tissues (Donoho, 1984).

Methods involving immunoaffinity chromatography and chemiluminescent ELISA, and liquid chromatography-electrospray mass spectroscopy, have been developed to detect monensin residues at nanogram concentrations (Blanchflower and Kennedy, 1996; Godfrey et al., 1997). Bioavailability, distribution, and depletion have been studied in chickens by injection and intracrop administration (Henri et al., 2009). Residues were not detected 6 h after withdrawal except in fat where the drug was detectable 12 h later. In another study, monensin was detected only in liver, kidney, and fat 24 h after the last dose. No residues were detected after 48 h, except in liver from which the drug had cleared by 72 h (Atef et al., 1993).

In the United States, no withdrawal period before slaughter is specified if monensin is the sole drug given to chickens at approved concentrations (Anonymous, 2009). A withdrawal period of 5 days is specified if used with various other drugs. In the EU, a withdrawal of 1 day is required. In practice, withdrawal periods used by the poultry industry are considerably longer. No residual activity was detected after withdrawal of monensin 24 hours before infection with Eimeria (McDougald and Seibert, 1998). Monensin is degraded in about 10 weeks in manure from cattle incubated at 37°C but more rapidly under natural weathering conditions (Donoho, 1984). In one study, the half-life in nonsterile soils was less than 4 days (Sassman and Lee, 2007). No effect was observed on 14 common crop plants when grown in soil treated with manure from cattle fed monensin (Donoho, 1984). Monensin can be detected in surface waters around agricultural operations but did not cause toxicity to freshwater macrophytes at environmentally relevant concentrations (McGregor et al., 2007).

**TOXICITY**

There have been several previous reviews of the toxic effects of ionophores (Braunius, 1985; Reece, 1988; Dowling, 1992). Although there are some reports of toxicity, years of commercial use have shown that monensin is safe if fed at approved dosage levels (Anonymous, 2006). Adverse effects can occur from errors in the calculation and formulation of medicated feed, inadequate mixing, and exposure of nonintended species to medicated rations. In the EU, carryover of monensin into unmedicated feed has been reported at mills with inadequate quality control (Kennedy et al., 1998).

General clinical manifestations of monensin toxicity include reduced feed intake, decreased growth rate and weight gain, depression, ruffled feathers, recumbency, ataxia, leg weakness, paralysis, and death (Novilla, 1992). Pathological indications include focal degenerative cardiomyopathy, skeletal muscle necrosis, and congestive heart failure. The onset of toxicity can be rapid, birds becoming drowsy and depressed within a few hours with death occurring in some birds a few days later. Signs are not pathognomonic and will vary depending upon many factors. A presumptive diagnosis of toxicity requires careful evaluation of circumstantial evidence and confirmation of the presence of high concentrations of drug in the feed (Anonymous, 2006). Monensin is believed by industry personnel to reduce feed intake at approved concentrations during warmer months of the year, but proof of this contention has not been provided. The effect of 121 mg/kg of monensin upon feed intake in chickens subjected to heat stress and infected with *Eimeria* species was investigated by Reid et al. (1976). In one experiment out of 9, in which birds were infected with *E. acervulina*, a reduction in feed intake was observed. No significant differences in mortality, weight gain, or feed conversion were found between heat-stressed chickens that were unmedicated or given 100 mg/kg of monensin (McDougald and McQuistion, 1980a). An interesting finding was that compensatory growth caused by increased feed consumption can occur during the first week after withdrawal of monensin from feed (McDougald and McQuistion, 1980b). Drug withdrawal had no effect upon feed conversion.

A variety of harmful effects of monensin have been described, but these are often anecdotal and in most
cases a lack of scientifically controlled studies makes interpretation difficult. One of the earliest examples was the belief that monensin promoted development of necrotic enteritis by slowing intestinal motility (McDougald et al., 1972). Experimentation proved this not to be the case. Monensin has been implicated in slow feathering in chickens (Kingston, 1977), particularly where sulfur-containing amino acids are borderline or deficient. However, no effect upon the rate of feather growth or the back-feather coverage was found when birds were given a nutritionally adequate diet (McDougald and Keshavarz, 1984). In another study, 175 mg/kg or more was required to adversely affect feathering (Yamane et al., 1982).

Potential cardiovascular effects of ionophores in mammals have been discussed by Pressman and Fahim (1983). High concentrations of monensin have toxic effects upon the cardiovascular system of chickens (Hanrahan et al., 1981; Wagner et al., 1983) and Julian (1993) has suggested that monensin toxicity may result in mortality of up to 5% in broiler flocks caused by pulmonary hypertension (ascites). No differences in mortality due to ascites and no differences in right ventricle:total ventricular weight ratios or electrocardiogram values were observed in chickens reared in floor pens and given 121 mg/kg of monensin, indicating that pulmonary hypertension did not occur more frequently in birds given this drug (Chapman et al., 1995).

OTHER AVIAN SPECIES

In the United States, monensin is approved for use in turkeys (59.5 to 99 mg/kg) and is used widely for this purpose. Medication usage for 5 feeds in turkey toms (provided during 0 to 3, 3 to 6, 6 to 10, 10 to 12, and 12 to 14 wk) is provided in the Agri Stats database. Data for March 2005 to 2009, the peak month of monensin use, indicate that the drug was employed by 25.3, 71.9, 89.3, 63.2, and 45.7% of complexes for the 5 respective feeds. Analysis of monthly 6- to 10-wk data for 2005 to 2009 is presented in Figure 6. As with chickens, a seasonal difference is evident, monensin being used primarily in winter and spring (December through May) with a decline through summer and fall (June through November). In the EU, approximately 97% of starter and grower feeds provided to turkeys contain an anticoccidial drug, but the extent of monensin use is not documented. Further information on the use of monensin in turkeys is available (Chapman, 2008).

Monensin is approved for use in bobwhite quail (80.5 mg/kg) and is effective in the ring-necked pheasant (Norton and Wise, 1981; Jurkovič et al., 1982; McQuiston 1987) and Japanese quail but not the chukar (Norton, 1986; Ruff, 1986).

Although most avian species of Eimeria develop in the intestinal tract, those that parasitize sandhill and whooping cranes have an unusual proliferative phase of the life cycle because they can develop in the respiratory tract and disseminate to the viscera, resulting in hepatitis, bronchopneumonia, myocarditis, splenitis, and enteritis (Novilla and Carpenter, 2004). Monensin, at a dietary concentration of 99 mg/kg, provided protection against this condition in sandhill cranes (Carpenter et al., 2005).

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