CHEMOTHERAPY OF ANIMAL PARASITES[1]

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Introduction

The past decade has been one of great activity in the field of chemotherapy of animal parasites; several excellent reviews of recent developments in the field have been published (1–3). This presentation will be concerned mainly with developments in the use of anthelmintics to control parasites of livestock, primarily nematodes, with emphasis on those of importance in ruminants.

The natural factors which contribute in some measure to the control of helminths in food-producing animals are numerous and diverse; some are inherent in the host-parasite relationship, i.e. the immune response of the host; others are environment-dependent, such as the physiological state of the host. Some of these factors will be discussed later; the important point to emphasize here is that the use of chemotherapeutic agents represents but one of many factors which must be considered if satisfactory results are to be achieved.

The attributes which characterize the ideal anthelmintic within the framework of this discussion are as follows:

Toxicity.—The toxic effect on the host should be minimal at the dosage level required. The attainment of this ideal can be extremely difficult.

Efficiency.—The drug must exhibit a high level of anthelminitc efficiency when it is used under natural conditions. This is not to say that absolute efficiency is a desirable goal in every case; other considerations such as the immune state of the host may be important.

Ease of administration.—The route by which a drug is administered may be a significant factor in determining its use. Individual restraint of animals in order to administer a drug orally or parenterally is a relatively costly procedure; for this reason, there is considerable interest in introducing the drug as a mixture of feed or water. This method of self-medication, however, involves many problems. Wide variations in consumption rates result in some animals ingesting enough drug to present a toxic hazard, while others will ingest so little that the anthelmintic effect is nullified.

Residues.—In recent years, particularly in the United States, there has been great concern over the hazards to human health which may accrue as the result of ingesting food containing small amounts of drugs, or their metabolites, which have been administered to control disease. It goes without saying that the ideal anthelmintic should not create problems of this nature.

[1] The survey of literature pertaining to this review was concluded in June 1967.

Cost.—The commercial production of animals for food is a highly competitive enterprise, one characterized by narrow profit margins. The producer will not automatically select the best anthelmintic in terms of efficiency; he will select a compound which will yield satisfactory results at a cost which will allow the attainment of a maximum profit margin.

ANTHELMINTICS

Although the development and testing of new compounds continues at a feverish pace, those which are in fairly common use someplace in the world are rather few in number. In this section, a selected group of anthelmintics will be discussed, which are being used to control gastrointestinal nematodes in ruminants. The selection is arbitrary and reflects the authors' opinions regarding those that are in wide use.

Phenothiazine.—Much remains to be learned about this versatile compound, although it has been in use as an anthelmintic for three decades (4). Evidence indicates that phenothiazine exerts its anthelmintic properties by coming in direct contact with the cuticle of the parasite (5). There is a direct relationship between the specific surface of the drug and its anthelmintic efficiency (6–8), and small amounts of impurities produce a disproportionate reduction in anthelmintic efficiency (9).

Depending upon the dosage rate, phenothiazine exhibits three types of activity. Small doses, of the order of 50 mg per kg, given over a period of time, will completely inhibit egg production in many nematodes. Similar doses will also produce a level in the feces which is toxic to the free-living stages of many nematodes (10). Therapeutic dose levels, of the order of 500 mg per kg, produce a direct toxic action on many nematodes in the gastrointestinal tract.

Phenothiazine may be administered as a bolus, in capsules, or as a drench with a wetting agent; however, the bulk of the dose required tends to make these formulations onerous and time-consuming, in practice. Attempts to avoid this problem by administering therapeutic doses in feed, often mixed with flavoring agents, have not proven very successful; such mixtures are often unpalatable, particularly to cattle, and give erratic results (11).

The toxicity of phenothiazine to ruminants has been extensively reviewed (2). Cattle appear to be somewhat more susceptible to toxic effects than sheep. Toxicity is increased as the phenothiazine particles become smaller with consequent increase of absorption through the wall of the gastrointestinal tract. Toxicity is also increased, as would be expected, in animals that are anemic or dehydrated.

Thiabendazole.—The anthelmintic properties of thiabendazole were first reported in 1961 (12). In ensuing years, it has been the subject of many reports and has become clearly established as the leading anthelmintic for the control of gastrointestinal nematodes of ruminants. Thiabendazole has a broad spectrum of anthelmintic activity against adult worms, including *Ostertagia*, *Haemonchus*, and *Trichostrongylus* in the abomasum, and *Tri-*

chostrongylus, *Cooperia*, *Nematodirus*, *Chabertia*, and *Bunostomum* in the intestine (13–15). Many workers have also reported significant activity against immature forms, particularly fourth stage larvae; unfortunately, most reports fail to differentiate between histotropic and lumen dwelling stages. It has been further observed that within one hour of administration of thiabendazole, the development of eggs passed in the feces was inhibited (16, 17).

The effective dose of thiabendazole in sheep and cattle varies between 50 and 100 mg per kg depending on the genera of helminths present. Dosage rates of 50 mg per kg have been found to be generally effective in sheep, while cattle require up to double this amount in order to attain satisfactory efficiency. Armour et al. reported that 110 mg per kg produced 64 per cent mortality of early fourth stage *Ostertagia* and was generally satisfactory for routine prophylaxis; however, in severe cases, they recommended doubling this dose (18).

Thiabendazole is very well tolerated by sheep and cattle; doses of 800 to 1000 mg per kg may produce signs of intoxication including depression and anorexia; doses of 1200 mg per kg may result in the death of sheep (13, 19).

Tetramisole.—The first report of this interesting compound appeared in 1966 (20); since that publication, a number of others have appeared which indicate that this drug is extremely promising for the control of gastrointestinal nematodes. Dosage rates of 15.0 mg per kg provide excellent control of adult *Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Nematodirus*, *Cooperia*, *Chabertia*, *Oesophagostomum*, and *Bunostomum* in sheep; however, unpublished data available to the authors suggest this dose can be halved for use against most of these genera in sheep and cattle. This dosage is only partially effective against *Strongyloides* and poorly effective against *Trichuris*. Immature worms appear to be equally susceptible with the exception of *Ostertagia* (21). Equally effective results have been reported in cattle against adult *Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Cooperia*, *Bunostomum* and *Oesophagostomum*. In terms of ease of administration, tetramisole approaches the ideal drug; it is effective when it is administered orally, intrarumenally, subcutaneously, or intramuscularly; however, tissue injection may produce an undesirable inflammatory response. Further attributes of tetramisole are that it is water-soluble and, because of its high efficiency, the bulk of the effective dose is minimal.

Methyridine.—First reported in 1961 (22), this drug has been the subject of extensive trials in sheep and cattle. It is effective when administered orally or parenterally; in the latter instance, it should be given interperitoneally or subcutaneously since intramuscular injections often result in severe abscess formation (23). Methyridine is more effective against helminths in the intestine than in the abomasum. This is probably due to the fact that its effectiveness is impaired in an acid environment; however, in practice this may not be as serious a deficiency as it suggests, since the pH of the abomasum is markedly increased in clinical ostertagiosis (24).

The standard dose of methyridine in sheep and cattle is 200 mg per kg; this level is very efficient in removing species of *Cooperia*, *Trichostrongylus*, *Nematodirus*, and *Strongyloides* from the small intestine. Action against *Haemonchus*, *Ostertagia*, and *Trichostrongylus* in the abomasum tends to be less effective and somewhat erratic.

One serious problem in the use of methyridine is its narrow margin of safety; therapeutic doses of 200 mg per kg have been reported to produce signs of intoxication in both sheep and cattle (25). When the narrow margin of safety, which necessitates a careful estimation of the weight of the animal to be treated is considered, the use of this drug would appear to be somewhat limited.

Ruelene.—This organic phosphorus compound is effective against many genera of nematodes in sheep and cattle. In sheep, doses of 100 to 200 mg per kg have been found effective in removing *Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Cooperia*, and *Bunostomum*. Indifferent results have been reported against *Nematodirus*, *Oesophagostomum*, *Chabertia*, *Trichuris*, and *Strongyloides* (26, 27).

In cattle, ruelene at dose rates of the order of 50 mg per kg have resulted in satisfactory activity against *Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Cooperia*, *Oesophagostomum*, and *Bunostomum*.

There is some evidence indicating that topical application of ruelene may exert anthelmintic and oogenic effects against some gastrointestinal nematodes in cattle.

Sheep appear to be somewhat more tolerant of ruelene than do cattle. The toxic dose for sheep is about 300 mg per kg as compared with as little as 60 mg per kg in cattle (28, 29). Although animals displaying signs of intoxication can frequently be treated satisfactorily with atropine and make an uneventful recovery, the very narrow margin between the effective anthelmintic dose and the toxic dose imposes a severe limitation on the use of ruelene.

Haloxon.—The anthelmintic properties of this organic phosphorus compound were first reported in 1962 (30). It has a broad spectrum of activity in both sheep and cattle. A dose rate of 50 mg per kg gives excellent control of *Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Cooperia*, *Bunostomum*, and *Stronglyloides* in sheep. Activity against *Oesophagostomum* is variable (17, 31). In cattle, dose rates of 50 mg per kg are highly effective against adult *Haemonchus*, *Cooperia*, *Ostertagia*, *Trichostrongylus*, *Nematodirus*, *Neoascaris*, *Oesophagostomum*, *Chabertia*, and *Trichuris*. Adult *Bunostomum* are refractory as are the immature stages of *Ostertagia*, *Oesophogostomum* and, to a lesser degree, *Trichostronglylus* (32, 33).

In contrast to many organic phosphorus compounds, haloxon has a relatively high therapeutic index; in sheep this ranges from three to four in aged ewes, to five to ten in young lambs. Cattle dosed at 100 mg per kg showed no signs of toxicity nor inhibition of red cell cholinesterase. High dose rates in sheep, of the order of ten times the therapeutic dose, may induce a neurotoxic response in certain animals; this takes the form of a hind leg ataxia.

Concepts and Considerations

Since the study of a medicinal is not complete until its performance under applied conditions is ascertained, it is appropriate to discuss certain concepts and considerations pertinent to the use of anthelmintics. Depending on the nature of the biological associations involved, the use of anthelmintics may be classified as strategic, tactical, therapeutic, and diagnostic (34, 35). These uses are dependent upon a clear understanding of certain fundamental concepts; if inadequate attention is given such concepts, even the most highly efficient anthelmintic may produce unsatisfactory results.

In the rational use of anthelmintics, it is essential that consideration be given to the complete life cycle of the parasite and to all the ecological and biological factors which make up the infection cycle. Fundamentally, all of the parasites with which we are presently concerned have similar and direct life cycles. The adult worms, females and males, are located in the gastrointestinal tract. After mating, the female deposits fertile eggs which are passed from the body with the feces. Following a period of embryonation, a first stage larva develops which hatches and actively feeds prior to moulting to the second stage larva. The second stage larva again feeds and undergoes a second moult to become the third stage or infective larva. The third stage larva, which has retained the cuticle of the second larva as a sheath, is actively motile but does not feed, and prior to any further development, must be ingested by an appropriate host. Upon ingestion, the sheath is cast off and the larva enters the mucosa of the abomasum or intestine initiating the histotropic phase of development. During this phase, a third moult occurs resulting in the fourth stage larva. The fourth stage larva may leave the mucosa and complete its development in the lumen or, more commonly, remain within the mucosa until the fourth moult occurs. This moult results in the early fifth stage "larva," which is the immature adult, this stage usually leaves the mucosa to become mature in the lumen. For the purposes of this presentation, it is much better to view this cycle in terms of an infection cycle consisting of four stages: (*a*) symbiotic, (*b*) contamination, (*c*) extraparasitic, and (*d*) infection. It is suggested that the cycle be considered analogous to a biochemical cycle in which the rate of the cycle is determined by the rate-limiting stage of the cycle. The ultimate action of an anthelmintic is the sum of its influence on these various stages. All efforts to control a parasitic disease, including those of the host, must be directed toward maintenance of the infection cycle at a rate commensurate with a commensalistic or mutualistic form of symbiosis. The alternative to this, if disease is to be avoided, is complete interruption of the cycle, which can be accomplished economically only in certain situations, such as in feedlots. In this instance, the level of extraparasitic development is greatly reduced and the infection stage essentially eliminated.

For the most part, anthelmintics are directed at the symbiotic stage. However, as in all cyclic systems, one cannot alter one stage without in-

fluencing other stages. As noted by Donald et al. (36), fitness for survival of an obligatory parasite ". . . may be defined as the net reproductive capacity of the total parasitic burden in a population of host/parasite realtionships." If this is true, one would expect such established evolutionary relationships as the gastrointestinal nematodes and ruminants to have attained some relatively stable biological balance, and indeed they have. *Haemonchus contortus*, which inhabits the abomasum, is relatively high in pathogenic potential, 3000 to 5000 adult worms being fatal to lambs, and in addition, individual female worms are relatively high in biotic potential, depositing some 5000 to 10,000 ova per day. This may be contrasted with *Ostertagia circumcincta*, which has a relatively low pathogenic potential, 10,000 to 30,000 adult worms are required to produce serious disease. This species has a relatively low biotic potential, each female producing some 300 to 600 ova per day. In order that both parasites might attain an appropriate fitness for survival, evolution has provided natural rate controlling factors at two stages in the infection cycle. In the symbiotic stage, the immune response is more effective in eliminating adult *H. contortus* than *O. circumcincta*. In the stage of extra-parasitic development, *H. contortus* is much more susceptible to adverse environmental conditions and has a shorter period of survival in the nonfeeding infective form. If such factors did not exist, the host population would soon be diminished to a point where survival of the parasite would be threatened. Man's intrusion into this evolutionary relationship with his husbandry methods, designed for maximal productivity, has so altered these stages that the danger of an increased cycling rate, with ultimate parasitic disease, is an ever-present threat to the livestock industry. With the advent of management practices in livestock production which are conducive to parasitic disease, the demand for anthelmintics has greatly increased. As indicated above, one must always consider the entire infection cycle if satisfactory results with anthelmintics are to be attained. For example, continuous prophylactic feeding of phenothiazine does not kill adult nematodes, but has a decided effect on fecundity and the development of free-living stages. This should directly influence the contamination and extraparasitic stages, while indirectly influencing the infection and symbiotic stages. Such use of this compound has met with considerable success against *H. contortus*, but results against other worms, of lower fecundity, have been less rewarding.

Symbiosis is generally subdivided into three types of animal associations. Mutualism, sometimes considered synonymous with symbiosis, is that association wherein both animals in the symbiotic relationship are benefited. Commensalism is that state in which one of the animal species is benefited, usually the smaller of the two, while the second is neither harmed or benefited. Parasitism is that association wherein one species, the parasite, may or may not benefit, but wherein the second species is harmed in some manner. As with all biological phenomena, there is never an absolute steady-state. Therefore, it is essential that one appreciate that even though an animal species is classified as a parasite, it may at some period in its symbiotic rela-

tionship assume any form of symbiosis. The importance of this concept is that the overall beneficial, or detrimental, effect of an anthelmintic will be determined by the existing form of the relationship and by the direction and rate at which it is changing.

The time at which an anthelmintic is administered is of great importance. In lambs, immunity to gastrointestinal nematodes does not appear to develop prior to 18 weeks in age (37). In some species of nematodes, early infection of lambs with small numbers of infective larvae promotes increased resistance in later life. However, if infection with large numbers of larvae occurs, there is a deleterious effect on the development of immunity in later life. This latter effect may be considered to be a form of immunological unresponsiveness resulting from exposure to excessive amounts of antigen. Examination of the infection cycle indicates several avenues of anthelmintic use relative to this phenomenon. The use of drugs which remove ova-producing nematodes from other animals on the pasture will tend to slow the rate of the cycling process. If such an anthelmintic has the additional property of inhibiting the development of extraparasitic stages, i.e., phenothiazine or thiabendazole, the cycle will be further slowed, reducing but yet maintaining exposure of the newborn.

One factor, presumably immunological in origin, which tends to control nematode populations is arrested or inhibited development of larvae in the symbiotic stage. The extent to which this phenomenon occurs over the wide spectrum of nematode species is not known, but it has been observed in *Ostertagia circumcincta* infection of sheep (38); *Trichonema* spp., in the horse (39); *Haemonchus placei*, in cattle (40); *Ostertagia ostertagi*, in cattle (41); *Nematodirus spathiger*, in sheep (36); and *Obeliscoides cuniculi*, in rabbits (42). These examples suggest that this may well be a fundamental characteristic of the symbiotic association. Sommerville (38) suggested that the extended histotropic period might influence the action of anthelmintics, since larvae of *Ostertagia circumcincta* in the gastric pits would, in all probability, be protected. Gibson (39) provided further and more ominous insight into the importance of this phenomenon when he demonstrated that the removal of a more or less stable population of adult *Trichonema* spp. from the intestine of horses allowed rapid development of the arrested or inhibited larvae. Roberts & Keith (40) subsequently demonstrated this same effect with *Haemonchus placei* in cattle. In this instance, the ability of cattle to develop a solid immunity as the result of repeated doses of larvae was demonstrated. However, when repeated therapeutic doses of phenothiazine were given during the immunizing period, the resulting immunity produced was reduced, and animals often acquired large populations of nematodes. Classic studies in Scotland with cattle further illustrated the importance of this phenomenon as it relates to pathogenesis and therapy of ostertagiosis (41).

The importance of the above studies relative to the use of anthelmintics is manifold. Drugs, such as phenothiazine, which appear to depend on direct contact with the nematode have little or no effect against the larval stages in

tissues. Drugs, such as thiabendazole, haloxon, and tetramisole, which have systemic as well as contact activity, can be expected to have potential action against these stages. Further complicating the chemotherapeutic picture is the fact that an individual drug may be quite active against adult worms of a given species and inactive against larval stages of that species, while in the case of other species, the reverse may be true. Thiabendazole, for example, at dose rates up to 67 mg per kg, is effective in removing mature *Ostertagia* from calves, but is not effective against immature forms; the reverse is true in the case of *Cooperia* (43). Herlich reported *Oesophagostomum* adults were effectively removed by thiabendazole, but larvae were practically unaffected. Both adults and larvae of *Trichostrongylus* appeared to be highly susceptible (44).

The foregoing illustrates several important aspects. When animals are continually being exposed, as on irrigated pastures, it is important that due regard be given to the nematode species of primary importance. Repeated use of anthelmintics with high efficiency against adult *Ostertagia* spp. may, in the case of animals recently placed in the infection cycle, prevent the development of clinical disease, but may also prevent the development of an adequate immune response. This would be due to factors analogous to those encountered by Roberts & Keith (40) with *Haemonchus placei*. If this occurred and the infection rate was high, then one could expect a continuing need for regular strategic and tactical use of anthelmintics. If, however, one can utilize an anthelmintic in such a manner as to allow the primary immune response to occur, yet prevent disease, it should be possible to move the symbiotic relationship to one of mutualism wherein the parasite is provided sustenance and the host is provided protection against subsequent exposure (36, 45). Ostertagiosis of cattle presents a particularly clear example of this concept. Armour & Urquhart (41) discussed outbreaks of Type I ostertagiosis, in which large accumulations of larve do not occur. This form can be expected to respond to most of the modern anthelmintics, since anthelmintics are quite active against adult nematodes of this genus. These same workers suggest this form of the disease can probably be controlled by strategic treatment since the occurrence of the disease is dependent upon a progressive build-up of adult nematodes over a period of at least nine weeks. Under management practices in Great Britain, it is therefore suggested that use of an anthelmintic in mid-June and again in early August will prevent the appearance of clinical Type I disease. It is noted that strategic treatment is dependent upon the rate of the infection cycle. In this regard, these workers point out that on less heavily infected farms, i.e., where a lower cycling rate is occurring, " . . . it is possible that a single treatment, preferably about the middle of July, might be sufficient. . . ." They further state that, " . . . on farms where the owner is prepared to rotate his calf-rearing pasture annually (not necessary on new leys), we think anthelmintic medication may be normally unnecessary. . . ." This, it is noted, would be a direct means of reduc-

ing the cycling rate, and even though as noted by Stoll (48), worm-host associations " . . . represent no opportunity for easy and permanent separation," such practice would allow the development of the immune response and maintain a commensalistic or mutualistic relationship.

Armour & Urquhart (41) state, "Anthelmintics are not successful in the treatment of established cases of Type II ostertagiosis; neither do they remove inhibited fourth stage larvae which accumulate during the asymptomatic period. . . ." In Great Britain, it has been established that inhibited larvae do not accumulate until after September and on this basis it has been suggested that the disease may be prevented by appropriate " . . . anthelmintic medication of susceptible calves in the middle of September followed by their removal to pasture not grazed in the same year by young stock" (41). While Type II ostertagiosis has not been reported in the United States, the authors have encountered a condition which they believe to be similar in pathogenesis, and in which the use of an anthelmintic may have been responsible for a serious disease outbreak. In this instance, a group of yearling steers, which had been on irrigated pasture from October to December, were to be moved to a winter foothill range where no cattle had been grazed since the previous May. On a rather large random sampling of animals, quantitative fecal examination revealed nematode ova counts ranging from zero to 350 per gm with a mean of 64 ova per gm. Examination of ova indicated they were primarily those of *Ostertagia ostertagi* and *Cooperia* spp. The animals were in good physical condition and even though the ova counts did not indicate heavy infection, it was decided to treat them with thiabendazole in order to reduce the potential contamination of the essentially uncontaminated range. They were treated (100 mg per kg) and moved to the range in the first week of December. Approximately three weeks later, severe gastrointestinal parasitism occurred simultaneously in all animals. Nematode ova counts in fecal samples collected at this time ranged from 50 to 1900 per gm, with a mean of 682 per gm. Autopsy of seven representative animals in February and March revealed the presence of 1690 to 7290 adult *Ostertagia ostertagi* in the abomasum, and 2128 to 43,795 adult *Cooperia* spp. in the small intestine. Since explosive outbreaks do not occur in Type I ostertagiosis, it must be concluded that one of two conditions led to this outbreak. The drug may have removed the adult nematodes and concurrently the factor(s) which were responsible for arrest or inhibition of larval development, thereby initiating further development of large numbers of larvae. Alternatively, the drug may have been ineffective and a true Type II disease occurred. In either case, one is led to conclude that Type II disease, or a very close approximation, does occur in the United States. This very useful classification of ostertagiosis should be extended to encompass epidemiology and pathogenesis of all nematodes exhibiting such phenomena.

As previously noted, thiabendazole exhibits good anthelmintic activity against immature *Cooperia* spp. at prophylactic dosage levels, but requires

much higher levels to attain satisfactory activity against the mature stages of this genus. It is quite conceivable that the lower dosage would be the more desirable in maintaining a state of commensalism or mutualism. Thiabendazole might be useful by allowing the primary immunological threshold to be reached (36, 45). This contrasts with the work of Roberts & Keith (40) with *Haemonchus* which showed that only adult worms were removed by the drug. In this case, primarily immature worms would be selectively removed.

In many instances, *Cooperia* spp. may be the primary pathogenic nematode present in cattle (47), and very often the population is predominantly in the adult stage of development (Type I). In selecting an anthelmintic for treatment of this condition, not only should efficiency and spectrum of activity be considered but the important factor of drug cost should be considered. While therapeutic dosage of thiabendazole will give good results, the organophosphates, tetramisole, and phenothizaine, will in many cases give equal or better results at relatively lower dosage and expense.

As noted by Chopra & Chandler (48), there has been a tendency, even from the earliest attempts to develop anthelmintics, to consider all parasitic nematodes alike, and thus efforts have been directed by the concept that only compounds with a wide spectrum of activity warrant consideration. The foregoing discussion indicates that such an approach is obsolete and, with increasing knowledge of symbiotic relationships, compounds must be found which have selective action against individual species, as well as against the various stages of development of the species.

The problem of differences in susceptibility to anthelmintics of nematode species has been mentioned. The problem of strain resistance within a species deserves comment as well. At present, only meager information is available on this problem, but there can no longer be any doubt that it exists and in all probability will assume greater importance. Foster (49) indicated there is little experimental evidence to suggest that therapeutic or prophylactic regimens of drug use have been responsible for the development of strain resistance to phenothiazine. The earliest attempt to demonstrate such a relationship was made by Sinclair (50) in *Trichostrongylus colubriformis*. While these results were negative, it should be noted that only six generations were exposed to phenothiazine. It is quite unlikely that, even had a resistant strain existed within the population, it would have been selectively isolated in so few generations. The chance of a spontaneous mutation being identified would be even more improbable. As noted by Kalow (51), drug resistance in bacteria can be due to tolerance, dependence, or destruction. He also noted that although the ability to destroy the drug might be gained by physiological adaptation, hereditary mechanisms were most important. It does not appear possible within the foreseeable future for parasitologists to apply refined techniques, such as used in bacteria to demonstrate conjugation, transformation, and transduction, even if analogous events occur in metazoan parasites. It does appear safe to assume that within populations of nema-

todes, there exist genetically resistant strains which may be dominant on initial exposure to an anthelmintic or which, under conditions of continued exposure, might selectively become dominant.

Drudge (52) confirmed the existence of a phenothiazine-resistant strain of *Haemonchus* on premises where phenothiazine had been used for a considerable period of time. Subsequently, such resistance has been demonstrated in strains isolated elsewhere (53, 54). The history of exposure of these strains to phenothiazine would suggest a causal relationship. However, the resistance to thiabendazole found in strains of *Haemonchus* on first exposure indicates that resistance was dominant in the population prior to exposure to this drug. In any case, the resistance is relative and would appear to be due to a genetically-mediated tolerance. Unfortunately, nothing is known of the biochemical mechanisms by which this tolerance is manifested.

The present authors have encountered a strain of *Ostertagia circumcincta*, in which an organophosphate eliminated only 40 to 50 per cent of adult nematodes, whereas a comparable dose of the drug against another strain eliminated 85 to 100 per cent. There was no possibility of previous exposure of the resistant strain to synthetic organophosphates, and it must be concluded that the strain was naturally resistant. Deduction indicates that the mechanism by which resistance occurred was either through drug tolerance or destruction, both of which must be genetic in origin.

Malone (55) demonstrated a neurotoxic response in some sheep given oral doses of haloxon at five to ten times the therapeutic level. Lee (56) found that two types of sheep existed, one in which *in vitro* hydrolysis of haloxon in plasma occurred rapidly, and one in which the hydrolysis was slow. Only sheep in the slow group were susceptible to the neurotoxic effect. Subsequently, Lee (57) demonstrated the presence of three types relative to the rate of haloxon hydrolysis, and six genotypes when additional substrates were utilized. The hydrolytic activity of plasma was attributed to plasma A-esterase. Tucker et al. (58, 59), studying the genetic inheritance of plasma esterases in sheep, observed an A-esterase in certain animals which protected them against the neurotoxic syndrome of haloxon. This A-esterase has been shown to be the same as described by Lee (57). Haloxon, as noted by Malone (55), is a rather unique organophosphate in that even at 10 times the therapeutic dose in sheep, only a slight to moderate depression of red cell cholinesterase occurs. In studies at the University of California, it has been found that even though the depression is not great, it is significantly greater and of longer duration in A-esterase-negative than in A-esterase-positive sheep.

Lee & Hodsden (60) and Lee (61) have presented convincing evidence that the selective activity of haloxon against nematodes is directly related to the irreversibility of the inhibited cholinesterase of most susceptible nematodes and the reversibility of the inhibition of sheep erythrocyte cholinesterase. With respect to organophosphate-resistant nematodes, it is quite

conceivable that a similar A-esterase may exist and may segregate with a totally unrelated factor, which in the course of evolution may lead to fitness for survival of the various strains existing in different environs. In addition, there is also the possibility that resistant strains may have cholinesterases which, as in sheep, are reversibly inhibited.

One is led to speculate on the role genetic variation in the host may play with respect to the anthelmintic action of chemical compounds. It is probable that much of the anthelmintic activity of haloxon is mediated by systemic means. If such is the case, it is highly probable that A-esterase in the plasma and tissues of the host may dramatically influence the ultimate anthelmintic activity. This would constitute one example simulating nematode resistance to an anthelmintic.

It has been suggested that criteria of the ideal anthelmintic include the properties of little or no toxicity to the host, killing all worms swiftly, and effectiveness in a small and easily administered single dose. There are significant exceptions to such criteria. Some of these exceptions have been mentioned where there are immunological considerations involved. Excluding the possibility of the development of resistant nematodes, there are many instances in livestock production where the continuous feeding of an anthelmintic would be of considerable value in controlling the infection cycle. To some extent, this has been achieved with phenothiazine, when fed as a prophylactic to reduce ova production of the existing populations of nematodes. If, however, the infection stage has reached a point where parasitic disease is likely to result, such a practice will be of little immediate value. In this instance, it would be far more profitable if the compound when fed continuously would either kill the adult nematodes immediately or have a cumulative action, wherein only a portion of the adult worms and larvae are killed each day, thus allowing more opportunity for acquired immunity to develop. Unfortunately, most anthelmintics in use at the present time behave much like phenothiazine and lose their vermicidal capacity when administered in multiple doses at less than the therapeutic level (62). Further complicating the application of anthelmintics to livestock is the problem of administration, particularly in pastured animals where repeated treatment is most likely to be needed. Phenothiazine and thiabendazole must be administered orally, while ruelene, although much more effective orally, does have some activity when applied to the skin (63). Other investigators, utilizing ruelene in the treatment of acute clinical gastrointestinal parasitism of dairy calves, observed a marked reduction in oogenesis of *Ostertagia ostertagi* and *Cooperia* spp., with no apparent clinical improvement or reduction in mortality when the chemical was applied topically. When it was administered orally, ruelene gave dramatic curative results (64).

Additional criteria of the ideal anthelmintic would include palatability and activity when fed in low dosage over extended periods; and cumulative activity such that if the animal does not ingest the desired amount each day, efficiency will not be adversely affected. No anthelmintic at present exists

which meets all these additional criteria. In this respect, the case of coumaphos suggests that producers of pharmaceuticals should not discard a compound with demonstrated action because of its failure to meet one of the "cardinal" criteria.

It has been known for some time that coumaphos is a very efficacious anthelmintic against the more important gastrointestinal parasites in the abomasum and small intestine of cattle and sheep (65, 66). Early in the developmental stages of this compound, it was found that the effective oral dose was dangerously close to the LD_{50}. As a result, little further attention was given the compound as an anthelmintic. Recently, coumaphos was investigated as an insecticide for fecal breeding flies when fed continuously at low levels. As part of the testing program, it was noted when coumaphos was fed at 1.25 mg per kg for a period of six or more days, there was a marked anthelmintic action. The authors conducted controlled tests in clinical gastrointestinal parasitism of cattle in which the compound was fed for six days at 2.0 mg per kg and obtained efficiencies of 85 per cent and 99 per cent against mature *Ostertagia* and *Cooperia*, respectively. From this, and other studies, coumaphos appeared to be cumulative in action, nontoxic when fed for long periods at the level of 1.25 mg per kg and relatively inexpensive when used for the required periods. Hopefully, coumaphos and other compounds with similar useful characteristics will soon be developed and approved for use.

It is impossible in this presentation to encompass all forms of management and factors which influence the rational use of anthelmintics in livestock. One particular type of management which can be singled out is the feedlot operation. In this instance, animals are placed in a corral and fed entirely in feed bunkers. This is the closest approach to the separation of parasite and host which can be attained in practice. From the chemotherapeutic standpoint, this represents only the symbiotic stage of the cycle. In this instance, if the animals need therapy, the sooner it is administered, the greater will be the benefits. Where treatment is warranted, the next decisions are the selection of the drug and method of administration. These decisions should be based partially on the species of nematodes present and the cost. Under such conditions, it is often desirable to add the drug to the ration either by top-dressing or as the ration is mixed. A fact often ignored is that even under feedlot conditions, animals will not consume the same amount of feed each day, even though total consumption over several days or weeks is comparable. Some compounds are unpalatable, and without special preparation, individual variation in consumption will be aggravated. As previously noted, most compounds tend to show a marked reduction in efficiency when used in less than a single therapeutic dose. With a compound such as thiabendazole, which has no palatability or toxicity problem, it is possible to feed more than the required therapeutic dose, or to feed the therapeutic dose for several days. In either case, the cost would be a major deterrent. An additional problem encountered in feedlot anthelmintic use is the fact that it is undesirable to incorporate a compound into the formulated ration unless it is

to be fed for some period of time. Thus, when an anthelmintic is to be fed for one day only, the usual procedure is to top-dress the compound. This practice increases labor costs and permits selective feeding and consequent variation of dosage between individuals. A compound such as coumaphos, which can be fed over the entire period animals are in feedlot, and which not only removes adult nematodes but would remove inhibited larvae as they emerge, would have obvious advantages. It is unlikely, however, that even at the cost of the lower dosage, such extended use for anthelmintic purposes could
might provide justification.

The authors cannot refrain from expressing their opinion relative to the widely held concept of "subclinical parasitism" and the recommendations, based on this concept, which aim to eliminate all nematodes from livestock. Even if this were possible, which it is not, we would not subscribe to it in all instances for reasons previously stated. It is true that with the advent of highly efficient anthelmintics, these medicinals can be used for diagnostic purposes (34). However, we do not subscribe to the use of the term "subclinical parasitism" in instances where otherwise undiagnosed disease occurs. Where measurable benefits accrue from anthelmintic use, irrespective of how these benefits are measured, the symbiotic relationship must have been one of parasitism, and was in no sense subclinical. Where no benefits accrue from the use of anthelmintics and nematodes were present, the symbiotic relationship must have been either commensalistic or mutualistic.

LITERATURE CITED

1. Elslager, E. F., Thompson, P. E., *Ann. Rev. Pharmacol.*, **2**, 193–214 (1962)
2. Gibson, T. E., *Veterinary Anthelmintic Medication* (Tech. Communication No. 33, Commonwealth Bureau of Helminthology, Farnham Royal, Bucks, England, 2nd ed., 206 pp., 1965)
3. Thompson, P. E., *Ann. Rev. Pharmacol.*, **7**, 77–100 (1967)
4. Harwood, P. D., Jerstad, A. C., Swanson, L. E., *J. Parasitol.*, **24** (Suppl.), 16 (1938)
5. Douglas, J. R., Baker, N. F., Longhurst, W. M., *Am. J. Vet. Res.*, **17**, 318–23 (1956)
6. Douglas, J. R., Baker, N. F., Longhurst, W. M., *Am. Vet. J. Res.*, **20**, 201–5 (1959)
7. Gordon, H. McL., *Australian Vet. J.*, **32**, 258–68 (1956)
8. Whitten, L. K., *New Zealand Vet. J.*, **4**, 63–68 (1956)
9. Baker, N. F., Allen, P. H., Longhurst, W. M., Douglas, J. R., *Am. J. Vet. Res.*, **75**, 409–13 (1959)
10. Taylor, E. L., Sanderson, K. M., *Vet. Rec.*, **52**, 635–47 (1940)
11. Baker, N. F., Allen, P. H., *J. Am. Med. Assoc.*, **133**, 265–68 (1958)
12. Brown, H. D., Matzuk, A. R., Ilves, I. R., Peterson, L. H., Harris, S. A., Sarett, L. H., Egerton, J. R., Yakstis, J. J., Campbell, W. C., Cuckler, A. C., *J. Am. Chem. Soc.*, **83**, 1764–65 (1961)
13. Bell, R. R., Galvin, T. J., Turk, R. D., *Am. J. Vet. Res.*, **23**, 195–99 (1962)
14. Baker, N. F., Douglas, J. R., *Am. J. Vet. Res.*, **23**, 1219–23 (1962)
15. Ames, E. R., Cheney, J. M., Rubin, R., *Am. J. Vet. Res.*, **24**, 295–99 (1963)
16. Southcott, W. H., *Australian Vet. J.*, **39**, 452–58 (1963)
17. Barnett, S. F., Berger, J., Rodrigues, C., *E. Africa Agr. Forestry J.*, **29**, 195–98 (1964)
18. Armour, J., Jennings, F. W., Kirkpatrick, K. S., Malezewski, A., Murray, M., Urquhart, G. M., *Vet. Rec.*, **80**, 510–14 (1967)
19. Gibbs, H. C., Pullin, J. W., *Can. J. Comp. Med. Vet. Sci.*, **27**, 3–8 (1963)
20. Thienpont, D., Vanparijs, O. F. J., Raeymaekers, A. H. M., Vandenberk, J., Demoen, P. J. A., Allewijn, F. T. N., Marsboom, R. P. H., Niemegeers, C. J. E., Schellekens, K. H. L., Janssen, P. A. J., *Nature*, **209**, 1084–86 (1966)
21. Ross, D. B. *Vet. Rec.*, **79**, 392–95 (1966)
22. Broome, A. W. J., Greenhalgh, N., *Nature*, **189**, 59–60 (1961)
23. Johnston, R. W., *Vet. Rec.*, **74**, 285 (1962)
24. Anderson, N., Armour, J., Jarrett, W. F. H., Jennings, F. W., Ritchie, J. S. D., Urquhart, G. M., *Vet. Rec.*, **77**, 1196–1204 (1965)
25. Groves, T. W., *Vet. Rec.*, **73**, 196–201 (1961)
26. Douglas, J. R., Baker, N. F., *J. Am. Vet. Med. Assoc.*, **135**, 567–69 (1959)
27. Enigk, K., Eckert, J., *Zentr. Veterinarmed.*, **9**, 165–84 (1962)
28. Herlich, H., Porter, D. A., Isenstein, R. S., *Vet. Med.*, **56**, 219–21 (1961)
29. Weidenbach, C. P., Radeleff, R. D., Buck, W. B., *J. Am. Vet. Med. Assoc.*, **140**, 460–63 (1962)
30. Brown, N. C., Hollinshead, D. T., Kingsbury, P. A., Malone, J. C., *Nature*, **194**, 379 (1962)
31. Berger, J., *E. African Agr. Forestry J.*, **29**, 199–205 (1964)
32. Hart, J. A., *Vet. Rec.*, **76**, 337–40 (1964)
33. Bosman, J., *J. S. African Vet. Med. Assoc.*, **36**, 251–57 (1965)
34. Gordon, H., McL., in *The Evaluation of Anthelmintics*, 90–102 (Merck & Co., Rahway, N. J., 180 pp., 1964)
35. Gordon, H. McL., *Australian Vet. J.*, **26**, 65–72 (1950)
36. Donald, A. D., Dineen, J. K., Turner, J. H., Wagland, B. M., *Parasitology*, **54**, 527–44 (1964)
37. Soulsby, E. J. L., in *Drugs, Parasites and Hosts* 62–76 (Little, Brown & Co., Boston, 367 pp., 1962)
38. Sommerville, R. I., *Nature*, **171**, 482 (1953)
39. Gibson, T. E., *J. Helminthol.*, **27**, 29–40 (1953)
40. Roberts, F. H. S., Keith, R. K., *Australian Vet. J.*, **35**, 409–14 (1959)
41. Armour, J., Urquhart, G. M., *Brit. Vet. J.*, **121**, 392–97 (1965)
42. Russell, S. W., Baker, N. F., Raizes, G. S., *Exptl. Parasitol.*, **19**, 163–73 (1966)
43. Rubin, R., Ames, E. R., Cheney, J. M., *Am. J. Vet. Res.*, **26**, 668–72 (1965)
44. Herlich, H., *Vet. Med.*, **58**, 874, 881–82 (1963)

45. Dineen, J. K., *Nature*, **197**, 268–69 (1963)
46. Stoll, N. R., in *Drugs, Parasites and Hosts*, 3–14 (Little, Brown & Co., Boston, 367 pp., 1962)
47. Alicata, J. E., *Am. J. Vet. Res.*, **21**, 410–15 (1960)
48. Chopra, R. N., Chandler, A. C., *Anthelmintics and Their Uses* (Bailliere, Tindall & Cox, London, 291 pp., 1928)
49. Foster, A. O., *J. Am. Vet. Med. Assoc.*, **139**, 490–91 (1961)
50. Sinclair, D. P., *Australian Vet. J.*, **29**, 13–17 (1953)
51. Kalow, W., *Pharmacogenetics*, 1–231 (W. B. Saunders Co., Philadelphia, 231 pp., 1962)
52. Drudge, J. H., Leland, S. E., Jr., Wyant, Z. N., Elam, G. W., Hutzler, L. B., *Am. J. Vet. Res.*, **20**, 670–76 (1959)
53. Enzie, F. D., Colglazier, M. L., Whitmore, G. E., Thompson, D. E., *J. Parasitol.*, **46** (Suppl.), 41 (1960)
54. Levine, N. D., Garrigus, U. S., *Am. J. Vet. Res.*, **23**, 489–93 (1964)
55. Malone, J. C., *Res. Vet. Sci.*, **5**, 17–31 (1964)
56. Lee, R. M., *Biochem. Pharmacol.*, **13**, 1551–68 (1964)
57. Lee, R. M., *Genet. Res. Camb.*, **7**, 373–82 (1966)
58. Tucker, E. M., Suzuki, Y., Stormont, C., *Federation Proc.*, **25**, 612 (1966)
59. Tucker, E. M., Baker, N. F., Stormont, C., Fisk, R. A. (Unpublished)
60. Lee, R. M., Hodsden, M. R., *Biochem. Pharmacol.*, **12**, 1241–52 (1963)
61. Lee, R. M., *Exptl. Parasitol.*, **18**, 332–37 (1966)
62. Douglas, J. R., Baker, N. F., Longhurst, W. M., *J. Am. Vet. Med. Assoc.*, **131**, 369–71 (1957)
63. Ciordia, H., Bizzell, W. E., Baird, D. M., McCampbell, H. C., *Georgia Agr. Expt. Sta., Mimeo. Ser.* **152**, 1–7 (1962)
64. Crenshaw, G. L., Baker, N. F. (Unpublished)
65. Herlich, H., Porter, D. A., *Vet. Med.*, **53**, 343–48 (1958)
66. Baker, N. F., Douglas, J. R., Crenshaw, G. L., Smith, E. M., *Vet. Med.*, **55**, 73–75 (1960)

CONTENTS