REVIEW ARTICLE

THE CHEMOTHERAPY OF HELMINTHIASIS*

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THIS review deals in the main with helminth infections of the gastro-intestinal tract of man and animals.

The control of helminthiasis in man and animals will not be accomplished through chemotherapeutic measures alone. Such measures are, of course, of immediate value but of supreme importance is the establishment of a high standard of hygiene to minimise spread of infection. Stransky and Reyes[1] quote that while human ascaris infection in Central Europe is not higher than 3·5 per cent, under more primitive conditions, in the tropics, the percentage of infestation may be as high as 90 or more. The establishment of satisfactory hygienic conditions in the animal field will be more difficult. However, other measures of control available to the farmer could include the use of ovicides, continual use of chemotherapeutic agents at a low level as prophylatics, and immunological measures[2].

The progress made in the chemotherapy of human and animal helminthiasis has been slow and the few successes achieved in recent years do not match those made in the bacterial and protozoal fields. Anthelmintics have been in continual use since the days of early man when it was recognised that a diseased condition was associated with infection by helminths. Whereas diseases such as trypanosomiasis, yaws, leprosy, malaria and others are slowly being brought under control in tropical areas helminthiasis is still as pressing a problem as it was 50 years ago. It is a sad reflection that the 2000 years-old filix mas treatment of tapeworm infection is still in use today despite the fact that it is not always successful and the drug has a very small chemotherapeutic index. Some of the older remedies and also some of the present day treatments, especially of the gastrointestinal infections of animals, can only be described as drastic. Substances like lead arsenate, sodium arsenite, nicotine sulphate, carbon disulphide, and carbon tetrachloride, are amongst the more noxious materials on the chemist's shelf. These treatments are possible under conditions where the quantity of drug absorbed into the blood system is small and activity against helminths is by virtue of the generally poisonous nature of the substance. Much larger chemotherapeutic indices are required for drugs for use in blood and tissue diseases. The aim should be, to develop chemical agents which function specifically as anthelmintics with chemotherapeutic indices larger than those of the majority of anthelmintic drugs in present day use.

Many helminth diseases exist for which no anthelmintic treatment is available or the available treatment yields variable results and is often hazardous.

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There are two main contributory factors which are probaby responsible for the slow pace of development of chemotherapeutic measures and which will be discussed in detail. The first of these has been the failure to recognise the harmful effects of the disease and the second has been the difficulty involved in devising satisfactory screening methods.

Is Helminthiasis of Importance?

Helminth disease of man and animals is seldom fatal. It lacks the urgency which is associated with more lethal diseases and it has consequently attracted less research effort from chemotherapeutists. Clinicians, however, now recognise that the health standards of whole populations are affected by helminthiasis, with a consequent lowering of resistance towards other diseases, and that heavy infection, especially of children inevitably leads to malnutrition. Jelliffe[3] states that infestation with the large roundworm, *Ascaris lumbricoides*, is almost universal in many tropical areas and that it is common for children to carry worm burdens of 100 or more. Stoll[4] has calculated that 644 million people are infected with *Ascaris lumbricoides* and 209 million people with the seat-worm *Enterobius vermicularis*. He estimates that 456 million people are infected with human hookworms, 39 millions with the beef tapeworm *Taenia saginata*, 20 million with the dwarf tapeworm *Hymenolepis nana*, 10 millions with the fish tapeworm *Diphyllobothrium latum*, 2½ millions with the pork tapeworm *Taenia solium*, and 27 millions with the pork trichina worm. Infection with blood and tissue worms or flukes is also as widespread.

The extent of infection in the animal world is even more striking. The farmer today is well aware of the damage to the economical and nutritional management of his stock. He realises that the insidious nature of the disease results in lower wool, milk and meat production. In 1937, the British Veterinary Association concluded that parasitic gastro-enteritis in sheep caused losses equal to £348,000 per annum in Great Britain. This figure could probably be multiplied by a factor of 3 according to the post-war value of sheep. A recent estimate by the Northern Ireland Ministry of Agriculture showed that liver fluke was responsible for the loss to them of approximately 360 tons of liver per annum. Foster[5] and Boughton[6] have made estimates of United States losses in the livestock industry through helminth infection. The latter quotes a financial loss of 227,672,000 dollars per year. He also estimates that swine in the United States carry a burden of 12 million lb. weight of *Ascaris lumbricoides*.

Difficulties of Screening

Gastrointestinal helminths in particular, exhibit not only host specificity but they also occupy specific localities within that host, depending on factors like oxygen tension, carbohydrate and vitamin supplies, pH, and nature of the host secretions at that locality. They are particularly sensitive to even small changes in the surrounding conditions and it has been possible only in a limited number of cases to culture helminths *in vitro*.

In vivo testing of related helminths in small animals is not entirely satisfactory as there is a wide variation in response of even very closely related helminths to the same anthelmintic. In the last analysis the drug must be tested against the particular helminth in the particular host. Such a screening was carried out on an extensive scale in man by Caius and Mhaskar[7] but these methods are time-consuming and expensive and must of necessity be limited in extent. However, *in vitro*, and *in vivo* screening tests in small animals, do allow a comparison to be made of the relative anthelmintic values of members of a series of compounds and comparisons with chemicals of known anthelmintic activity can always be made. The tests described below are some of those which are in general use. A more detailed discussion of screening methods and techniques of assessing individual activities of drugs is given by Stewart[8]. Whitten[9] discusses screening methods for compounds with taenicidal activity.

Test A. Trendelburg[10] found that the common earthworm responded to santonin and he then employed it as a screening test for human *Ascaris*. Although many investigators have pointed out the inadvisability of using annelid material for anthelmintic screening purposes, the test is still used. Singh and others[11] state that it may be of particular value as a preliminary screen for taenicidal action.

Test B. Hall[12] tested the activity of drugs against *Ascaris*, hookworm, trichuris and tapeworm in dogs by means of his "critical test". This test has been the basis of subsequent screening techniques using various host animals in which the numbers of worms voided after administration of drugs and numbers of worms remaining on post mortem are counted. Leiper[13] uses domestic hens infected with *Ascaridia*, *Heterakis* and *Capillaria*.

Test C. Lamson and others[14] used intact specimens of pig *Ascaris* which were kept alive for a limited period of time. In this test santonin was inactive.

Test D. Baldwin[15] has used tied off neuromuscular preparations of *Ascaris*. Although the majority of the known anthelmintics had an effect on the neuromuscular apparatus, phenothiazine and gentian violet were found to be inactive. Baldwin's test has been adapted by Chance and Mansour[16] for screening compounds active against liver fluke and by Batham[17] for the screening of compounds with taenicidal activity.

Test E. Whitlock[18] and Rogers[19] have used the trichostrongylid worm *Nippostrongylux muris* for the screening of potential anthelmintics for use in trichostrongyle, *Ascaris* and hookworm infections.

Test F. Erhardt[20] has used cats infected with strongyloides, whipworm, *Ascaris*, cestodes and trematodes for screening purposes.

Test G. Erhardt and Gieser[21] used rabbits infected with *Passalurus ambiguus* for the screening of oxyuricidal compounds.

Test H. Leiper[22] and Vanne[23] have used the free living vinegar eelworm for preliminary screening of potential nematocidal drugs.

Test I. Mice infected with *Aspiculuris tetraptera* or *Syphacia obvelata* or with both worms have been used for screening purposes[24–29]. The test is of particular value for screening compounds with oxyuricidal properties.

Test J. Stewart[8] suggests that a mixed infection of *Heterakis spumosa* and *Nippostrongylus muris* in rats could be a valuable screening test for activity against *Enterobius*, strongyles, hookworms and trichostrongyles.

Test K. Taenicidal activity has been specifically screened by Harwood and Jerstad[30] using *Raillietina cesticillus* in chickens and by Holton[31] using *Hymenolepis diminuta* and *H. nana* in mice.

Test L. Parnell[32] and later Levine[33] have used fresh horse strongyle larvae for the screening of compounds with larvicidal activity. Since this activity is directed towards nematode tissue Levine has expanded the test and has adopted it as a general screening test for anthelmintic activity to which known anthelmintics respond. Parnell and Mackie[34] have tested a large number of chemicals by this method.

But even if a particular chemical were known to have the required anthelmintic properties it cannot be said with certainty that anthelmintic action will follow in the intended host. Some helminths are well protected by mucous and some are more deeply embedded in the gut wall than others and so less prone to attack. Physical factors may therefore determine whether anthelmintic action takes place. These factors can also so modify anthelmintic action that a compound inferior in a screening test, might function better in the host than a more active compound owing to less interference from mucous or greater ease of penetration of the helminth cuticle. The rate of penetration of cuticle by hexylresorcinol, for instance, can be greatly diminished in the presence of mucin and bile salts and can be increased in the presence of small concentrations of a natural soap or a synthetic detergent[19,35,36].

In the assessment of results obtained from screening tests and in the consideration of whether any particular compound is likely to show activity in the host, such physical factors as the relation of solubility and absorption of the drug by the host to the locality of the helminth within the host, particle size of insoluble drugs, solubility and pH dependence, and behaviour in presence of mucous must be taken into account. Chemical behaviour, like possible hydrolysis of the drug, is equally important.

The establishment of satisfactory screening methods and indeed the solution of the problem of the chemotherapeutic approach to helminthiasis will be possible only through the co-ordinated efforts of biochemists, parasitologists, chemists, and veterinary or medical clinicians. Their problems in the helminth field are inter-related.

The recent findings in nutritional and metabolic studies of helminths, lead one to anticipate the establishment of screening tests in which it will be possible to test the action of chemicals against an organism in a habitat approaching that of its natural surroundings. Indeed, such studies might eventually lead to a more rational approach to chemotherapy where one could make attempts to interfere with essential metabolic processes of the helminth. But, while vermicidal activity might well be demonstrated in such *in vitro* tests, vermifuge action would be a more difficult matter to recognise.

Nutritional studies have established that utilisation of glucose and the rapid synthesis and utilisation of glycogen are the common function of

many helminths. Read and Rothman[37,38] and Read and Laurie[30] have studied the effect of diets rich and deficient in glucose and other sugars on the normal development of the tapeworm. Gaafar and Ackert[49] studied the effects of low calcium and phosphorus diets on the growth of *Ascaridia galli* in chicks. Other studies include the effect of exclusion from diet of vitamins A and B complex, proteins and minerals, on helminth growth and the resistance of the host animals to helminth infection[41–43].

The availability of such modern techniques as partition chromatography, radioactive tracer and electrophoretic methods has resulted in increased knowledge concerning the metabolic processes and chemical composition of helminths. The advances in this field have been reviewed by Bueding[44] amongst others, and more recently by von Brand[45]. Helminth oxidative mechanisms are inefficient and a variety of carbohydrate oxidation products results. Bueding[44] states that whereas human *Ascaris* excretes mainly the lower fatty acids, notably *n*-valeric acid, *Moniezia expansa* of sheep and *Fasciola hepatica* excrete mainly the higher fatty acids. Protein and lipid characterisation and metabolism in parasites have not received as much attention as have the carbohydrates. It is known that nematode cuticle is almost completely composed of protein. Recently, Bird[46] has found that the cuticles of three species of nematode *Ascaris lumbricoides* var. *suis*, *Toxocara mystax* and *Strongylus equinus* contain the majority of naturally occurring amino acids and that they show a similarity of number and type in the different helminths. There were, however, slight quantitative differences. The unsaponifiable lipid fraction in many parasites is large. Fairbairn and Jones[47] have confirmed the presence of cholesterol and saturated sterols as well as a substance related to ascaryl alcohol in the body wall of *Ascaris*.

Compounds Used as Anthelmintics

A large number of chemical compounds has been shown to exhibit anthelmintic properties to a widely varying degree. There are possibly more chemical agents available for combating helminth disease of the gastrointestinal tract than there are for combating any other type of disease. This is because a direct method of attack on such helminths has been possible, by the easy method of administration *per os*, and assessing the results by examining the faeces. These chemical agents may be large in quantity but they are poor in quality and relatively few are satisfactory anthelmintics from the point of view of efficiency, or chemotherapeutic index.

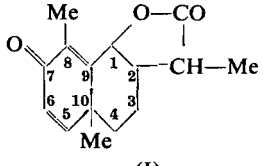
In the course of this review a selection of the accepted and more promising anthelmintics has been made.

The older anthelmintic remedies from plant extracts although sometimes exhibiting a high efficiency, frequently give rise to toxic symptoms. The chemist has been able to extract and characterise the active principles in these remedies so that an exact dosage schedule has been made possible. But variations in the structures of these active materials have not yet produced compounds of improved anthelmintic quality.

The natural products will be considered first.

Santonin

Santonin (1) is the active principle of *Artemesia maritima*, var. *anthelminticum*. Until quite recently it was the drug most widely used in the treatment of human ascariasis. Trendelburg[10] recognised that its anthelmintic properties were probably due to its effect on contraction of worm muscle.

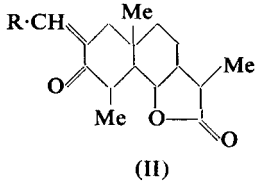


(I)

Caius and Mhaskar[7] tested a number of santonin-like compounds (santoninic, santonic and santonous acids, *desmotropo*santonin and santonone) in humans and concluded that the active centre of the santonin molecule was the ketonic grouping at 7. Baldwin[48] using Test D, in a study of similar santonin-like compounds found that the structures common to the active compounds of the series were an intact γ-lactone ring, a double bond at position 7, and an angular methyl group at position 10. He then proceeded to test a variety of compounds containing the active centres contained in santonin. Activity was shown in substituted alkyl and substituted phenyl acetophenones, various benzophenones and benzylidene acetones amongst which were found compounds with activities approaching those of thymol and β-naphthol. Alkylated resorcinyl ketones, cyclic diketones and α-ketoacids showed little promise.

Lautenschläger[49], von Oettingen[50] and other workers had previously found an activity amongst the lactones and this was confirmed by Baldwin[48]. He found that the γ-butyrolactones were particularly active but not as active as santonin itself and that coumarin and 3-coumaranone exhibited some activity. Recently Nakabyashi and others, have expanded this series. They have studied the relative anthelmintic activities of santonin and coumarin derivatives including octahydro- and thia-derivatives[51] and have concluded that the ketonic character of santonin is of supreme importance.

Cocker and McMurray[52] suggested that the active santonin compounds investigated by Baldwin were all capable of chelation and might function therefore by removing essential metallic ions from enzyme systems of nematodes. They however found little activity in a series of α-tetrahydrosantonin compounds (II) which contained the essential features for activity of the santonin molecule and also were capable of chelation.



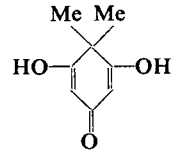
(II)

R = OH; C_6H_5; *p*-MeO·C_6H_4; CH_2—O

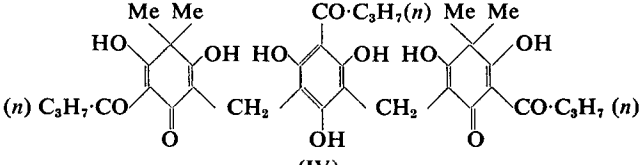
Filix Mas

An ethereal extract of male fern is a preparation widely used for treatment of tapeworm infestation in many animals and also in liver-fluke infestation of ruminants. Its chemical characteristics were first studied by Boehm[53]. The compounds present—filixic acid, aspidin, albaspidin, flavaspidic acid, filicinic acid, aspindol and others—are inter-related and are all phloroglucinol derivatives. Much investigational work has been carried out on the constitution and synthesis of these substances.

A frequently occurring degradation product is filicinic acid (III), which Robertson and Sandrock synthesised in 1933[54]. Flavaspidic acid and albaspidin as a by-product were synthesised by Riedl[55], from butyrylfilicinic acid, formaldehyde and butyrylmethylphloroglucinol. Recently Chan and Hassall[56] have proposed an alternative structure to that suggested by Boehm[57] for filixic acid, which is the major biologically active constituent. The formula they propose (IV), $C_{36}H_{44}O_{12}$, is in better agreement with the experimental analysis and is also in agreement with ultra-violet absorption data.



(III)



(IV)

The whole extract of male fern is still used since it is computed that each constituent contributes towards the anthelmintic activity. Anthonen[58] is of the opinion that pure flavaspidic acid is more effective and less toxic than male fern extract.

Inagaki and colleagues[59], have synthesised various acylphloroglucinols, methylphloroglucinols and their condensation products with formaldehyde with the view to testing their anthelmintic power. A large number of ketonic phloroglucinols has been synthesised by Riedl and others[60].

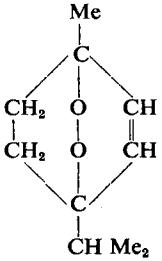
Of great interest is the fact that other tapeworm remedies of natural origin contain phloroglucinol derivatives usually involving a methylene bridge. Such substances are kamala which has been resolved by Khorana and Motiwala[61] into five constituents of which rottlerin and *iso*rottlerin alone showed anthelmintic activity. Birch and Todd[62] have proposed formulae for protokosin, α- and β-kosins which are found in "Kousso". These structures again contain the phloroglucinol unit.

Oil of Chenopodium

The oil from *Chenopodium anthelminticum* (American wormseed plant) was used for anthelmintic purposes by the South American Indians and was introduced into Europe in 1881. Its active constituent, ascaridole, is present to the extent of 45–70 per cent. Bruening[63] recognised it as a

valuable agent for removal of human *Ascaris* and hookworm but he also noted its toxic properties. It was formerly widely used for removal of ascarids from pigs but it has now been superceded for this purpose by the piperazines.

The structure of ascaridole (V) was elucidated by Nelson in 1911[64] and it has been synthesised by photochemical oxidation of α-terpinene by Bodendorf[65] and later workers.



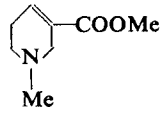
(V)

Caius and Mhaskar[7] reported that reduction of ascaridole increased its anthelmintic powers but its toxicity was thereby also increased. Little investigation of the anthelmintic activity of compounds similar in type to ascaridole has been made. A review of ascaridole and its uses has been given by Schenk[66].

Arecoline (VI)

This alkaloid is the principle constituent of areca nut. It has been used in the form of a variety of salts as an effective taenicidal agent, particularly for dogs.

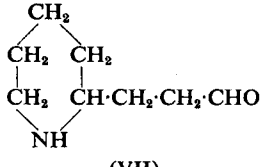
It was first used for this purpose by Lentz[67]. It frequently causes vomiting. The antidote for arecoline poisoning is atropine. Many derivatives have been synthesised, mainly during the earlier work in the elucidation of its structure but their taenicidal activity has not been recorded.



(VI)

Arecoline causes violent peristaltic movements and causes an outpouring of mucous secretion. Its taenicidal action may therefore be purely mechanical in that it causes the tapeworm to become detached and hence expelled from the host.

Similar taenicidal compounds are the pelletierines which occur in pomegranate bark. Four alkaloids are known to be present, viz. pelletierine (VII), *iso*pelletierine, methylpelletierine and pseudopelletierine. These compounds are toxic. Variations in the structures may prove of value in attempts to decrease their toxic nature.



(VII)

Synthetic Compounds

The Phenols

Phenolic substances have consistently been shown to exhibit anthelmintic properties usually of a nematocidal nature. In many instances the anthelmintic activity has paralleled the antibacterial activity. This has led to the belief that the phenols (as also do certain antibiotics) destroy the bacterial flora upon which the helminth may be dependent or with which it is in biological equilibrium. However, direct action by the phenols on the helminth itself has been recorded[14,35]. It is possible that phenols form complexes with essential metabolites through hydrogen bonding.

The anthelmintic properties of phenols were first recognised by Bozzolo in 1879[68], who used thymol for treatment of hookworm infestation.

β-Naphthol was used by Bentley in 1904[69] and further evaluated by Schüffner and Vervoort[70] in hookworm disease. Lamson and others[71,72], investigating the anthelmintic properties of phenols chose hexylresorcinol since this compound had been shown by Leonard[73] to be the most active germicidal agent of the alkylphenols. They found the compound to be 93–96 per cent efficient against *Ascaris* in man and that it was relatively non-toxic although it caused temporary irritation of the mouth. Lamson and others[14], then made a systematic study of more than 150 phenols, using screening Test C in the hope of finding a phenol which would show the same or better anthelmintic properties as hexylresorcinol, and yet be non-irritant. Despite this and much subsequent work, hexylresorcinol remains the phenolic compound of greatest activity against *Ascaris*, *Enterobius*, hookworm and *Trichuris*. It is also reported to have some taenicidal action.

The compounds examined by Lamson and colleagues[14] included the alkylphenols and alkylpolyhydric phenols, chlorinated phenols, naphthols, phenanthrols, hydroxydiphenylmethanes and ethanes, *cyclo*hexyl, benzyl and phenylphenols, etc. They found that in an alkyl series of phenols the amyl, hexyl and heptyl compounds showed maximum activity. In such a series they correlated anthelmintic activity solubility, and melting point of the phenol.

Other phenols of interest which have been announced from time to time are 4-*t*-butyl-2-chlorophenol[74], 2-ethyl-4-chloro-6-hexylresorcinol[75], 4-fluoro-2-propylphenol[76], 6-*t*-butyl-1-chloro-2-naphthol as a taenicidal agent[77], resorcinol ketones as taenicides[78], 1:8-dihydroxyanthraquinone, active against *Trichuris* in dogs[70] and *Trichuris* in sheep[89], 1-bromo-2-naphthol[81] and the hydroxydiphenylmethanes, which are discussed later. Tomita and others[82], in a study of active, chlorinated alkylphenols found all the compounds to be irritant to the tongue. Martin[83] could find no marked relation between structure and anthelmintic action in a series of polyhydric phenols. Fushimi[84] from a study of a large number of phenols established that the pig *Ascaris* screening test (Test C) was of value in the screening of compounds active against human *Ascaris*.

The irritant properties of hexylresorcinol can be greatly reduced by complex formation with piperazine[85] which itself is an active ascaricide (see later). Two compounds are described which are formed in the ratio of 3 piperazine:2 hexylresorcinol molecules and 1 piperazine:2 hexylresorcinol molecules. Both compounds readily break down in presence of acid to regenerate the components.

Phenolic compounds have proved of great value in the chemotherapy of helminthiasis. They are of especial interest in that they show a wide spectrum of activity and are relatively nontoxic. Investigational work into the anthelmintic activity of phenols is by no means a closed chapter and further work may yet result in improved anthelmintics.

Halogenated Hydrocarbons

This group of compounds displays anthelmintic activity particularly

against hookworm and liver fluke and to some degree against *Ascarids*. They are probably all protoplasmic poisons.

Hall[12] found carbon tetrachloride to be very efficient in hookworm infestation of dogs. Tetrachlorethylene was found by Hall and Shillinger[86] to be less effective than carbon tetrachloride but less toxic. Hexachlorethane[87–89] is another drug of this type which is now widely used for treatment of fascioliasis in cattle and it is claimed to be well tolerated by animals. Kudicke and Weise[90] in an examination of a number of halogenated derivatives of the lower hydrocarbons found that the anthelmintic activities were inversely related to the solubilities of the compounds. In general the bromo- compounds were more active than the chloro-analogues. Butyl chloride was found by Wright and Schaffer[91] in their study of chlorinated alkyl hydrocarbons to be highly active against *Ascarids* and hookworms in dogs and by Harwood and others[92], to be moderately active against whipworm in dogs. Its activity against whipworms, hookworms and roundworms of dogs was confirmed by Whitney and Whitney[93].

Other chlorinated hydrocarbons reported to show good *in vitro* activity are 4-hydroxy-4-trifluoromethyl-1 : 1 : 1 : 7 : 7 : 7 : hexafluoroheptane[94] and hexachloropentadiene[95].

Few halogenated aromatic hydrocarbons have been tested for anthelmintic properties. Dickmans[96] showed that *p*-dichlorobenzene was active against hookworms, roundworms and whipworms in dogs but Daubine[97] found it to be inactive against sheep helminths. Gordon[98] however, found that it was active against *Haemonchus contortus* and *Trichostrongyles* of sheep if the oesophageal groove reflex were first stimulated by a dose of copper sulphate. He found that *o*-dichlorobenzene was superior to the *p*-isomer and that bromo-, trichloro- and tetrachlorobenzenes were inactive.

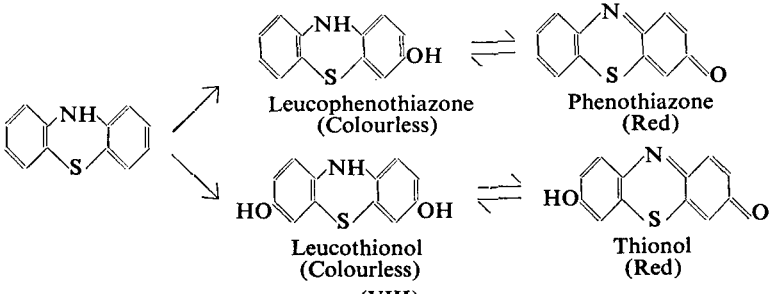
Phenothiazine

The discovery in 1938 by Harwood and others[99], of the valuable anthelmintic properties of phenothiazine proved to be a major contribution to the field of veterinary medicine. Although it is used for the treatment of helminth infection of many animals it is of particular value in nematode infections of sheep and cattle.

Several reviews dealing with the use of phenothiazine as an anthelmintic have appeared[100–103]. The compound is only slightly soluble in water (1 part in 800,000) yet 40 per cent of an oral dose is absorbed and excreted in the urine in the form of a number of soluble phenothiazine derivatives[104]. The metabolism of phenothiazine in the animal body has received considerable study but it has not yet been conclusively established whether phenothiazine itself or a derivative thereof is the active anthelmintic principle.

Phenothiazine is readily oxidised according to the following scheme[100] and such an oxidation-reduction system could conceivably interfere with essential enzymatic processes in the worm. Phenothiazine and its oxidation products are effective inhibitors of many mammalian enzyme systems[105]. It seems probable that phenothiazine causes paralysis of the

muscular systems of helminths and causes failure in the reproductive system. Lazarus and Rogers[196] using ^{35}S labelled phenothiazine found that the main portal of entry of the drug was via the helminth cuticle. Esserman[107] also using ^{35}S labelled drug, found that the intestinal fluid of rats and chickens fed with the labelled drug and also the parasites themselves (*Ascaridia galli*) contained phenothiazine and a complex of phenothiazine with a fatty substance and no oxidation product was detected.



(VIII)

Habermann and Shorb[108] showed that small daily doses of phenothiazine given to sheep in the form of a salt-lick were effective in inhibiting the development of worm larvae in the faeces. This technique of dosing has been examined with great thoroughness and it is now employed as a valuable prophylactic measure in the control of helminthiasis.

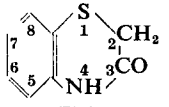
Although of unquestionable value to the farmer, phenothiazine has its limitations. The nematodes of sheep vary in their susceptibility to the action of the drug so that large doses are required for elimination of the more resistant helminths and some species of sheep helminths are totally resistant. Staining of wool by the coloured oxidation products is a serious problem in wool-producing areas.

Despite much chemical work involving the manipulation of the phenothiazine skeleton and formation of phenothiazine derivatives no compound with anthelmintic properties superior to those of phenothiazine itself, has been forthcoming. In general, it can be said that substitution in the phenothiazine molecule has the effect of decreasing the anthelmintic action. Activity is retained, however in those compounds, such as 10-acyl derivatives which can regenerate phenothiazine under *in vivo* conditions. *In vitro* testing of such compounds would indicate that they were inactive. Stable substituents in positions 3, 7 and 10 could reasonably be expected to yield compounds of diminished activity since these would prevent quinone formation (see VIII). An even greater reduction in activity which is unexpected, occurs when substituents are placed at positions 2 and 8 (Leiper and Watkins, unpublished).

Diphenylamine, xanthone, phenothioxine, and phenazine, even in large doses did not give promising results in limited tests against *Ascaris* or *Oesphagostomum* in swine but diphenylamine had some action in sheep, although it proved to be very toxic[109]. Guthrie[110] found that diphenylamine was active against *Trichuris* in dogs. Gordon and Lipson[111] found

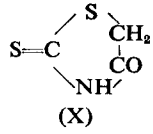
that phenothiazone and thionol were inactive but that phenothioxine had some activity against *Haemonchus contortus* in sheep. Gordon[112] also found that methylene blue, xanthone, trimethylthianthrene were inactive and Whitten[113] found the sulphoxide to be against *H. contortus.*

Mackie and Raeburn[114] found that phenothiazone was lethal and that thionol and phenothiazine sulphoxide were paralysant only in the Chance and Mansour screening test for liver fluke. They found considerable paralysant activity in a series of 6-substituted 2:3-dihydro-3-keto-1:4-benzothiazines (IX).



(IX)

Mackie and colleagues[115,116] tested a series of β-10-phenothiazinylpropionic acid esters and salts and found that a number had a paralysant effect on liver fluke but only the free acid and its sodium salt had any effect on roundworm (Test D). Derivatives of rhodanine (X) were also tested but little activity was obtained in the series. The benzylidene compounds however, had marked activity against liver fluke.



(X)

Rogers and others[117], using Test I have examined the anthelmintic activity of a number of tricyclic compounds in which the NH and S groups of phenothiazine were replaced by other groups such as CH, CO, N, O, S, Se, SO_2. They also tested carbazole and compounds in which its NH grouping had been replaced by other groups, diphenylamine and diphenyl sulphide. The only compounds which they found to be active were phenothiazine and phenoxazine. Other compounds prepared by these workers were 2:3-dihydrobenzo-1:4-thiazine and various alkyl-phenothiazines.

Levine (Test L) has examined various 10-substituted phenothiazines, phenothioxine, xanthone, xanthydrol, phenazine and phenoxazine. The potassium salt of 1:3:7:9-tetrasulphonic-5:5′-phenothiazine dioxide showed an interesting activity. (2:8?)-Phenothiazinedisulphonic acid as the soluble calcium or sodium salt has been reported by Pegreffi and Quesada[118] to be active in a bronchopneumonia caused by larvae and eggs of *Synthetocaulus rufescens.*

The Piperazines

The screening of compounds for filaricidal activity by Hewitt and others[119], using cotton rats infected with *Litomosides carinii* led to the discovery of Hetrazan, also named diethylcarbamazine, 1-diethylcarbamyl-4-methylpiperazine, hydrochloride. This compound which is now widely used in the treatment of filariasis has also been reported to have some action against *Ascaris* in man[120] and also against the *Ascarids* of dogs and cats[121,122].

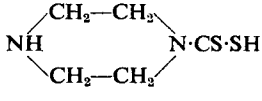
In 1949, Fayard[123] reported that the parent base piperazine was active against *Ascaris* in man. Mouriquand and others[124], found that it was active against *Syphacia obvelata* and *Aspiculuris tetraptera* in mice (Screening Test I) and they extended their studies to *Enterobius* and *Ascaris*

infections in man with successful results. Subsequent work has confirmed the value of piperazine and its salts in ascarid and oxyurid infections of man and animals[125–139].

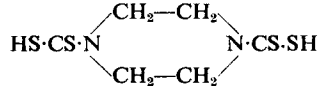
Gordon[131] showed that the piperazines were of limited use only, in helminthiasis of sheep. Marquardt and Fritts[132] found that there was no action against a variety of helminths in sheep.

Brown and colleagues[133] from tests in mice (Screening Test I) and Leiper and Watkins (unpublished) from tests in chickens, have shown that substitution in any position in the piperazine molecule has the effect of decreasing anthelmintic activity. This is reminiscent of the effect on activity, of substitution in the phenothiazine molecule. Harfenist[134], however, has found that some high alkyl substituted piperazines have an activity equal to that of piperazine itself.

Piperazine, which is a strong base, exists as a deliquescent hexahydrate. It is presented on the market in the more palatable form of salts (diphenylacetate, adipate, citrate, tartrate, dilaurate, etc.). Leiper[129] describes an insoluble complex of piperazine and carbon disulphide, which is decomposed by gastric juice into its components, both of which are anthelmintic in action. This compound was shown by Dunderdale and Watkins[135] to be a polymer structurally composed of the units, piperazine-1-carbodithioic acid (XI) and the piperazine salt of piperazine-1 : 4-dicarbodithioic acid (XII).



(XI)



(XII)

Oelkers[136] has noted that piperazine exerts a paralysant action on *Ascaris lumbricoides* and *Enterobius* and also on nerve preparations of earthworms and leeches. It acts as a vermifuge and worms are expelled alive from the host. Norton and Beer[137] find that piperazine produces paralysis of *Ascaris* by blocking the neuromuscular junction.

Piperazine salts in general are very soluble in water and Harned and others[138], have shown that a large proportion of an oral dose of piperazine is excreted in the urine. The high activity against *Ascarids* and particularly against *Syphacia obvelata* and *Enterobius vermicularis* is therefore surprising as it has been generally accepted that chemical agents with low water solubility are more likely to reach the localities of the intestinal helminths. It would be interesting to establish whether the piperazine susceptible helminths are affected by the direct action of possibly only a small fraction of the oral dose which reaches them or whether in fact it is the absorbed material in the blood stream, either as piperazine or a metabolite thereof, which is the active anthelmintic principle. It is interesting to note, in this connection, that phenothiazine which is virtually insoluble in water is particularly active against the sheep stomach worm, *Haemonchus contortus*, which has been conclusively shown by Leiper and Watkins (unpublished) using ^{32}P labelled blood, and by other workers to be

a voracious blood sucker. Here also it is possible that these particular worms receive their dose of drug through the blood stream which is known to contain phenothiazine metabolites. These observations may lead one to speculate whether more attention should be paid to administration of anthelmintics by parenteral methods.

Metallic Compounds

Copper sulphate and sodium arsenite are materials which have been of use in the treatment of sheep helminthiasis. Sodium fluoride[139] was formerly used for removal of *Ascarids* from pigs. Tin compounds also show interesting anthelmintic properties. Guthrie and others[140], showed that tetra*iso*butyl tin was active against *Raillietina cesticullis* in chickens and Guthrie and Harwood[141] showed that tin oleate, tartrate and oxalate were active taenicides. The taenicidal activity of "Stannoxyl"—a mixture of tin, tin oxide and tin salts was examined by Le Gac[142] and by Hirte[143]. Kerr and Walde[144] tested a large number of tin compounds against *Ascaridia galli* and *Raillietina cesticillus* in chickens. The compounds were of the type $R_{4-n}SnX_n$ where R = alkyl, aryl or aralkyl, X = O, S or an inorganic or organic anion and n = 1, 2 or 3. This work was followed by a report by Kerr[145] in which it was stated that di-*n*-butyl tin dilaurate was an efficient taenicide in chickens, of chemotherapeutic index = *ca.* 20. Kerr and Walde[146] have extended their study of tin compounds. They found a consistent activity against *A. galli* and *R. cesticillus* in 112 compounds of the type R_2SnX_2.

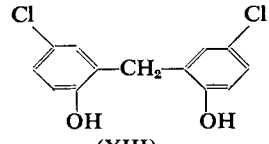
Compounds of the type R_3SnX were also tested and of these tributyl tin-acetate, -chloride and -thiol were found to be very active. Compounds of general formula $RSnX_3$ and R_4Sn were generally inactive. During the course of this work the authors made the observation that the compounds were likely to show a greater anthelmintic efficiency when they were administered to chickens in their feed than when they were administered in capsules. They concluded that distribution of a compound in the gut and possible repeated exposure to a compound is essential for a high degree of activity.

Cadmium compounds were shown by Guthrie[148] to be effective ascaricides in pigs but the soluble compounds proved to be rather toxic. However, cadmium anthranilate, being insoluble and therefore less likely to be absorbed from the gastrointestinal tract, showed less evidence of toxicity. A range of cadmium compounds was investigated by Levine and Ivens[149] (Screening Test L).

Diphenylmethanes

An interesting taenicidal activity has been discovered in halogenated hydroxydiphenylmethanes which compounds also exhibit germicidal properties. Craige and Kleckner[150] showed that 5:5′-dichloro-2:2′-dihydroxydiphenylmethane (XIII) (Dichlorophen, Diphenthane-70, G4) was very active against the tapeworms of dogs, and this compound is now widely used in the taenicidal treatment of a number of animals. Another compound of this series which also shows taenicidal action is

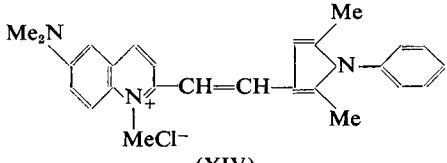
3:3′:5:5′:6:6′-hexachloro-2:2′-dihydroxydiphenylmethane[151] (Hexachlorophene, G 11). The compounds are efficient and not very toxic but the chemotherapeutic doses are large. The worms are killed by the drug and they then disintegrate during their passage through the intestines. A large number of diphenylmethane compounds have been synthesised as potential germicides but the anthelmintic properties of the majority are not known. Kerr and Green[152] studied a series in which the number and positions of chlorine and bromine substituents and of the two hydroxyl groupings were varied and the methylene grouping was also replaced by oxygen. Taenicidal activity apparently increased with increasing halogen content. It was not established whether the presence of hydroxyl groupings was essential for activity.



(XIII)

Cyanine Dyes

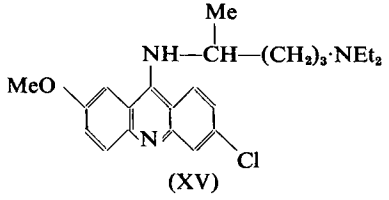
Various cyanine dyes have been shown to possess anthelmintic action. 6-Dimethylamino-2-[2-(2:5-dimethyl-1-phenyl-3-pyrryl) vinyl]-1-methylquinolinium chloride (XIV) was shown by Hales and Welch[153] to be active against *Ascaris*, hookworms and whipworms in dogs. It is also reported[154] that it is as active as the piperazines against *Enterobius vermicularis* in man. Cyanine compounds which have been reported to be of value in the treatment of helminthiasis of sheep and of threadworm in man are various 1:1′-dialkyl-2:2′-quinocarbocyanine salts[155], (1-alkylquinoline-2) (3′-alkylbenzthiazole-2′) trimethine cyanine salts[156] and 1:1′-dialkyl-2:2′-benzthiazole trimethine cyanine salts[157].



(XIV)

Acridines

Culberston[158] in 1940 found that mepacrine (XV) hydrochloride was active against *Hymenolepis fraterna* in mice and Neghme in the same year found that it was active against *Taenia saginata* in man. The compound is now widely used as a taenicidal agent in man and most of the reports of its use are favourable although toxic symptoms occur occasionally.

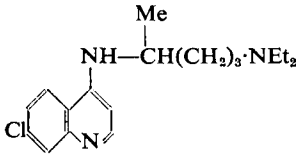


(XV)

Mustakillo and Saikonnen[160] suggest that mepacrine (an electropositive dye according to Keller[161]) abolishes the electrochemical forces responsible

for the attachment of the scolex of the worm to the intestine. The worm is usually expelled alive.

Camero[162] found that chloroquine (XVI) diphosphate was active against *Taenia saginata* and that it was non-toxic.



(XVI)

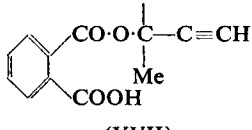
Surrey and others[163], found that compounds whose structures contained hydroxyl groups in the basic side chain of mepacrine had marked anthelmintic activity against *Aspiculuris tetraptera* and *Syphacia obvelata* in mice (Screening Test I). Kotova[164] and Semenova[165] have tested a large number of acridine compounds against *Hymenolepis nana* in mice. 5-Aminoacridine and 5-substituted-aminoacridines were reported to exhibit the greatest activity.

Antibiotics

A large number of antibiotics has been screened for anthelmintic activity, mostly by the mouse test (Screening Test I). The compounds active in such screening tests were—oxytetracycline[166], chlortetracycline[167,168], bacitracin[29,169], erythromycin[170], puromycin (Stylomycin) [171,172]. Some anthelmintic activity has also been found in neomycin, chloramphenicol but not in dihydrostreptomycin. Wells and others[173], found that oxytetracycline was active in *Enterobius vermicularis* infection of man and it is now widely used for this purpose. Hygromycin has been found to be active against *Ascaris lumbricoides* var. *suum* in pigs[147,174]. Puromycin has been reported by Young and Freed[175] to have some anthelmintic effect in *Enterobius vermicularis* infection of man.

Proteolytic Enzymes

Gastrointestinal helminths are able to withstand the action of digestive enzymes. *Ascarids*, for instance, produce an antienzyme, "ascarase" which has an anti-trypsin and anti-pepsin activity, so protecting the helminth cuticle from damage. In extracts of body wall of *A. lumbricoides* a chymotrypsin factor has recently been found which is different from the anti-trypsin factor from the same source[176]. However, a few proteolytic enzymes from plant products are known which are effective in digesting parasites. Examples of these are, ficin, from leche de higueron[177]; papain, from the paw-paw fruit[178]; raigan (*Omphalia lapidescens*)[170] and bromelin[189], from pineapple juice.



(XVII)

(XVIII)

Two recently introduced drugs which have anthelmintic properties of a specific character are 3-methyl-1-pentyn-3-yl sodium phthalate (Whipcide)

(XVII)[181] which is claimed to be very active against whipworm in dogs, and cyanacethydrazide (XVIII)[182] which may prove to be of value in the treatment of lungworm infestation of animals.

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