CONTRIBUTION TO THE CONTROL OF FASCIOLIASIS

STUDIES ON THE VARIATION IN NUMBER OF *FASCIOLA HEPATICA* EGGS EXCRETED BY CATTLE AND SHEEP, WITH APPLICATIONS TO THE SCREENING OF FASCIOLICIDES

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I. INTRODUCTION

Fascioliasis is one of the most important diseases, from the economic point of view, of cattle and sheep in the Netherlands. Economic losses result from the unthriftiness, diminished milk production, condemned livers, inefficient food utilization, and probably also from predisposition to other diseases. The impossibility of keeping sheep on many farms because of heavy liver fluke contamination of the pastures, constitutes a further, indirect cause of loss. The annual losses due to decreased meat and milk production and condemned livers, as a consequence of fascioliasis in cattle, were assessed by DOEKSEN *et al.* (1949) to amount about *f* 24,000,000 in the Netherlands. According to a recent estimate by VINK (1961), infections with *Fasciola hepatica* during 1959 caused a loss in this country of about *f* 204,000,000.

Since liver fluke infection cannot be contracted without the presence of the intermediate host *Lymnaea (Galba) truncatula*, the control of this snail seems to offer an attractive method for the prevention of infection in live-stock.

The eradication of snail populations from wet areas is probably best achieved through improved drainage. This is, however, not always possible or economically feasible since snail infested areas frequently cover a large area of the available pasture surface.

Another method of controlling the intermediate host is the application of molluscicides. Several chemicals have been recommended for the destruction of snails. Whatever molluscicide is used, long-term control of the snails always remains very difficult for the following reasons:

1. The area to be treated requires careful inspection by a specialist in snail ecology in order to determine its limits. This is time-consuming and expensive, and in many cases an inspection will be impracticable. The alternative is to treat large proportions of the area, and often huge surfaces, because one is not able to localise the habitats of the snails. This also results in high costs.

2. Spraying or broadcasting of molluscicides has to be carried out accurately in order to treat every small area where snails may be present. This is very difficult to achieve.

3. It is frequently difficult to prevent the introduction of snails from adjacent areas.

On account of these factors it is often impossible to eradicate snails by chemical means. One has to be content with obtaining a temporary reduction in the snail population by application of molluscicides on the areas which are supposed to be habitats of the snails. This operation has to be repeated at least once a year.

Apart from the control of the intermediate host, it is possible to adopt alternative

methods of interrupting the life cycle of *Fasciola hepatica* e.g. the prevention of infection of snails by chemotherapeutic control of the liver fluke in the final host. In order to be able to stop the excretion of liver fluke eggs in the faeces of cattle and sheep, drugs have to be available which are highly effective in killing the mature liver flukes. However, reports on the efficacy of the available fasciolicides are conflicting. Moreover, the safety of the usual treatments often appears to be unsatisfactory.

This publication presents the results of investigations on the chemotherapeutic control of *F. hepatica* infections in cattle and sheep. A new method was developed to test fasciolicidal activity of preparations. The value of some of the available drugs was re-assessed, and a search was made for new drugs, which would be sufficiently effective to be of value in the prophylaxis of fascioliasis, harmless for the host as well as for the consumer of animal products, and which would also be attractive from the economic point of view.

II. REVIEW OF LITERATURE ON MEDICATION OF INFECTED ANIMALS

1. MISCELLANEOUS DRUGS

There have been many attempts to find drugs capable of killing *F. hepatica* in the host, but only a few compounds have been discovered which appear to be of value.

It became possible to treat sheep and cattle more or less effectively after the discovery of the activity of male fern preparations. Although, according to MONTGOMERIE (1926), critical tests on the value of male fern in the treatment of liver rot of sheep were reported in 1884, this drug came into field use only about 50 years ago. Extracts of male fern have been used for many years, especially as constituents of a number of proprietary preparations (e.g. "Distol"); but their use has gradually decreased because of reports of toxicity and unreliable action. These preparations are difficult to standardize, and their anthelmintic activity often diminishes rapidly on storage.

MAROTEL (1931) reported results obtained with a proprietary preparation, "térébenthine-benzol". After experiments with 35 sheep and 10 cattle had shown that the fluke eggs in the faeces disappeared in every case in three or four days following treatment, field trials on a large scale were carried out in different parts of France. MAROTEL concluded that the treatment was very efficacious, and that the drug caused mortality in about 1 per 350 animals treated. The preparation, which is, according to NEVEU-LEMAIRE (1936), of Bulgarian origin, is still sold in France as "Vitan". According to Euzeby (1958, personal communication) analyses have shown that the composition of "Vitan" is approximately as follows: oil of turpentine 12%; benzene 78%; spirit of camphor 5% and petroleum jelly 5%. LEDERMAN (1958) reported that all cattle treated by him in the former Belgian Congo with "térébenthène-benzol" continued to excrete fluke eggs in the faeces, even if young animals were treated with two doses at intervals of one or five days. Faecal examinations were performed five or seven days following treatment. This author reported that in the region in question "Distomum hepaticum" occurs; this is probably *Fasciola hepatica* Linnaeus, 1758. *Fasciola gigantica* Cobbold 1885, however, can be expected to occur in that region, and it is therefore not clear with which species the treated animals were infected.

Reports on remedies against human fascioliasis have been reviewed by LÄMMLER (1955) and DESCHIENS (1958). Some of the drugs which have been tried appear to have provided cures: glucantime, antimony potassium tartrate, stibosan, anthiomaline, gentian violet, emetine hydrochloride with or without stibophen, and "rose de Magdala". These drugs have – to the writers knowledge – never been introduced for the treatment of fascioliasis in farm animals. LÄMMLER (1957) has tested a few of the

mentioned drugs in rabbits infected with *F. hepatica*. He found that stibophen and anthiomaline had some effect, but, because of their toxicity long courses of treatment would be required. Antimony potassium tartrate appeared to be less effective, and emetine hydrochloride was found not to possess anthelmintic action towards *F. hepatica* in rabbits. Antimony potassium tartrate was found by MURLASITS (1953) to have a slight effect on liver flukes in sheep, but the drug appeared to be too toxic to be used for the treatment of fascioliasis in these animals. HIRSCHLER (1957) found emetine hydrochloride, when administered orally to a sheep at the dosage rate of 2 mg/kg on each of three consecutive days, to have no effect on the excretion of *F. hepatica* eggs in the faeces. However, emetine hydrochloride is always administered parenterally in the treatment of fascioliasis in man. DESCHIENS (1958) recommended daily doses of 40 to 80 mg (about 1 mg/kg/day), given by intramuscular injection, for 8 to 10 consecutive days. The series of injections may be repeated some weeks later. According to DESCHIENS the treatment is considerably less effective against sexually mature flukes in the bile ducts than against immature flukes.

Carbon tetrachloride and hexachloroethane are the only drugs which are commonly used at the present time for the medication of sheep and cattle. The use of these compounds is therefore discussed in detail.

2. CARBON TETRACHLORIDE (CCl_4)

This drug has been used almost exclusively for sheep since about the year 1930 because early reports indicated its toxicity for cattle. WILSON & BLAKEMORE (1930) for example reported that cattle can die from only 5 ml given orally, and that dose appeared to be insufficient to kill all the flukes. An extensive review of experiences with the treatment of cattle has been given by GRAWERT & EICHMANN (1930). These authors treated liver fluke infected cattle with doses of 2.55 g (1.6 ml) of chemically pure CCl_4 per 100 kg body weight. The CCl_4, emulsified in water either with fatty or with mineral oil, was administered per os. *Fasciola* eggs were found about 26 days after treatment in the faeces of 66% of the animals. It is possible that immature flukes were present in the cattle when they were treated, and that some of these had become sexually mature when the faeces were examined 26 days later. Treatment of two experimentally infected calves at Weybridge with an oral dose as high as 5 ml per 100 lbs. body weight of CCl_4, mixed with liquid paraffin, was nearly completely effective against the mature liver flukes (MIN. OF AGRIC., 1961). Both animals seemed a little unwell the first 24 hours after treatment.

SPREHN (1932) stated that in 1924/25 he had found 1 to 2 ml CCl_4 to be completely effective when administered to sheep. MONTGOMERIE 1926*a*), who worked in Wales, found that an oral dose of 1 ml chemically pure CCl_4, given in soft gelatin capsules, was sufficient to kill all mature and almost mature liver flukes in sheep. This dose

was well tolerated by the experimental animals. MONTGOMERIE's findings were confirmed by de BLIECK & BAUDET (1927) in the Netherlands. OLSEN (1948) found in the U.S.A. that CCl_4 was not always completely effective against mature liver flukes. He gave 1 ml of chemically pure CCl_4, prepared commercially in soft gelatin capsules, to each of five fluky sheep. The percentages of flukes destroyed in the five sheep, three to six days after medication, ranged from 58 to 100%, with the average of about 90%. The treatment was completely effective in only one sheep. No immature flukes were present in these animals. By treating sheep which he had experimentally infected, MONTGOMERIE (1928) was able to show that the "assailable age" of the liver flukes becomes progressively less as the quantity of the drug was increased. With a dose of 1 ml CCl_4 immature flukes, less than eight weeks of age, remained alive, while a dose of 10 ml killed nearly all flukes which were at least five weeks of age. DEMIDOV & POTEKHINA (1959) found that a dose of 2 ml CCl_4 killed the flukes in sheep only when at least 11 or 12 weeks had elapsed between the administration of encysted metacercariae and treatment.

MONTGOMERIE concluded that in order to avoid the risk of intoxication it was not advisable to exceed doses of 1 ml for sheep. It has appeared, however, that an oral dose of only 1 ml, although generally perfectly tolerated by sheep, is not always safe. Soon after the introduction of the treatment in England MONTGOMERIE (1927) reported about four instances in which dosage with pure CCl_4 had produced deaths in certain flocks. Heavy losses in flocks of sheep, medicated with 1 ml of CCl_4 have been described by CLOUGH (1928, 1936) and by BLAKEMORE & MCDOUGALL (1946).

It is generally recognized that some flocks are considerably more susceptible than others. MONTGOMERIE (1926*a*) found that 50 ml of CCl_4 was perfectly tolerated by a sheep seriously affected by liver flukes, and de BLIECK & BAUDET (1927) experienced no fatality when they treated several sheep with 40 ml per animal. It has been reported that sheep and cattle receiving concentrated food are particularly susceptible (NORRIS, 1927).

Several factors have been found which influence flock susceptibility. CCl_4 was found by MINOT & CUTLER (1929) to produce severe intoxications in dogs on a meat diet which was low in calcium, while the addition of calcium salts to the meat diet or the feeding of a diet of bread and milk caused a high degree of tolerance to the drug. Cases of poisoning could usually be cured by calcium therapy. The authors concluded that for the safe use of CCl_4 a diet rich in calcium and carbohydrates should be given, and that foods which tend to give rise to increased guanidine in the blood should be avoided. NAKAMURA *et al.* (1958) found that rats fed a low protein diet followed by CCl_4 poisoning revealed a significantly lower incidence of liver necrosis and kidney injury than rats fed a high protein diet. GALL (1955) found a decrease of serum calcium content in sheep of 23% and in cattle of 15%, 24 hours after oral administration of CCl_4. According to CLOUGH (1936), HALL & SHILLINGER have found in dogs, and DAUBNEY in sheep, that administration of magnesium sulphate reduced the toxic

effect of CCl_4. An indication has been found by MUTH (1960) that an insufficient uptake of selenium by sheep makes these animals very susceptible to CCl_4.

Recent investigations into the pathology and prophylaxis of poisoning by CCl_4 have been discussed by GALLAGHER (1961) and REES *et al.* (1961) According to GALLAGHER CCl_4 damages semi-permeable membranes by its lipid solvent action. As a result liver cells, and probably also cells of other organs, lose cytoplasmic constituents, and in a later stage, mitochondrial components are lost. Cell death was considered to result from failure of respiration by the loss of pyridine nucleotides from the mitochondria. A number of drugs have been found to be of value in protecting animals against the toxic action of CCl_4: nicotinic acid, tryptophane, "Phenergan", vitamin E, selenium and diphenyl-p-phenylenediamine. Nicotinic acid and tryptophane seem to act by stimulating the synthesis of pyridine nucleotides, thus permitting the rapid replacement of lost co-enzymes. The remaining drugs were considered to prevent the loss of pyridine nucleotides from mitochondria. The protective effect of nicotinic acid in sheep was described in detail (GALLAGHER, 1960). The varying susceptibility of sheep to CCl_4 poisoning has also been discussed by GALLAGHER (1961) with reference to experimental studies. He stated: "Anything tending to increase the permeability of mitochondrial membranes should increase susceptibility to CCl_4 poisoning. Deficiencies of vitamin E, selenium or the balance of tissue reducing substances may be factors leading to greater susceptibility of sheep in the field. Hydrocortisone, the major adreno corticosteroid secreted by sheep, has been shown to increase mitochondrial permeability to pyridine nucleotides, so environmental or nutritional stress might well be a factor in determining susceptibility to poisoning by CCl_4."

NÖLLER & SCHMID found as long ago as 1927 that CCl_4 was effective against flukes in the bile ducts of sheep when injected either subcutaneously or intraperitoneally, and GANSLMAYER (1944) also reported that subcutaneous injection of this drug was effective in sheep. Nevertheless oral administration has become the common practice for many years, probably because periodic treatment of great numbers of animals could not be carried out by veterinary surgeons, and a treatment had to be chosen and advocated which could be applied by the farmers themselves. Some years ago, however, the interest in the possibility of parenteral application of CCl_4 was renewed.

WINTERHALTER & DELAK (1953) found that CCl_4 was less toxic to rats on subcutaneous injection than on oral administration. These authors (1954) obtained the same results with swine. CAMOU (1953) observed no intoxications after s/c[1]) injection of CCl_4 in about 500,000 sheep and goats and about 30,000 cattle in North Africa. In the following years the s/c injection of a few ml of CCl_4 was found to be highly effective against liver flukes in sheep by several authors (DEMIDOV, 1954; KURTPINAR, 1955; SLANINA *et al.*, 1955; WINTERHALTER & DELAK, 1955; STANIVUKOVIĆ, 1957;

[1] s/c: subcutaneous.

EHRLICH *et al.*, 1958; EHRLICH & WINTERHALTER, 1958; MEKULI & MARINČEVIĆ, 1958; ŠIMŮNEK, 1958; ULLRICH, 1958; SCHMIDT-HOENSDORF, 1959; CERNI *et al.*, 1959). It seems that often about 90% of the infested sheep were freed of mature flukes: DEMIDOV (1954), EHRLICH *et al.* (1958), EHRLICH & WINTERHALTER (1958), MEKULI & MARINČEVIĆ (1958). BORAY (1956) found *Fasciola* eggs in the faeces of 80% of the sheep four weeks after treatment by s/c injection of 2 ml CCl_4, and GNEDINA *et al.* (1958) reported that s/c injection of 3 ml CCl_4 per sheep reduced the number of flukes by 86.7%, while five out of ten animals were cured. Contrary to the mentioned observations, GAVEL' *et al.* (1958) and SEN'KOV (1958) reported that s/c injection of CCl_4 in sheep has no effect on liver flukes. VITUSHINSKII (1958) also stated that s/c injection of CCl_4 is ineffective.

Most authors have not reported serious intoxications after s/c injection of CCl_4 in sheep, but there are also different records. SLANINA *et al.* (1955), who treated 620 sheep with 2 to 3 ml of CCl_4 by s/c injection, observed excitement, giving way to apathy and inappetence during the first days following injection. Six of the sheep died, and of 36 pregnant ewes three aborted. Signs of intoxication were also seen by ŠIMŮNEK (1958), who concluded that the treatment should not be recommended because of the side effects. Heavy losses among sheep, treated by s/c injection of CCl_4, have been experienced by KAZAKOV & ZOTOV (1955), by GAVEL' *et al.* (1958), and by SEN'KOV (1958). ULLRICH (1958) and SCHMIDT-HOENSDORF (1959) employed the preparation "Ecobol", which contained CCl_4 in an oil in water emulsion at the concentration of 500 mg/ml. No harmful effects were observed by these authors when the preparation was injected at the dose rate of 0.4 ml/kg. BEHRENS (1960*a*), however, observed severe disease in five flocks of "Merinofleischschafe" two to three days after s/c injection of "Ecobol", and a number of the animals died. LÄMMLER (1956), who worked with rabbits, found that the chemotherapeutic index, i.e. the maximum tolerated dose divided by the dose which effectively removed mature *F. hepatica*, is considerably lower with s/c injection of CCl_4 than with oral administration.

KAZAKOV & ZOTOV (1955) did not see undesirable reactions after i/m[1] injection of the same drug in sheep. This mode of administration has been applied in sheep also by several other authors: BORAY (1956), EGYED & NEMESÉRI (1957), DELAK & MARŽAN (1959), EDWARDS (1959), PARRY (1959) and YAKOVENKO (1959), who all claimed to have obtained satisfactory results. Six weeks after i/m injection of 1 ml CCl_4 per 10 kg body weight BORAY (1956) found liver fluke eggs in 10% of the treated sheep. EGYED & NEMESÉRI (1957) injected 4 ml CCl_4 intramuscularly in 180 infested sheep weighing 30 to 35 kg, and after the treatment they found fluke eggs in the faeces of only 10 animals. The faeces were examined two weeks after treatment.

The risk of severe intoxication after i/m injection of CCl_4 in sheep seems generally to be very small. EDWARDS (1959), however, has reported losses amounting 0.5%

[1] i/m: intramuscular

after i/m injection of 3 ml CCl_4 in sheep. On the other hand, treatment with the same dose by mouth resulted in higher losses: 1.75% of the sheep died.

The encouraging results obtained by parenteral use of CCl_4 in sheep naturally led to similar studies on cattle. SLANINA *et al.* (1955) reported that s/c administration of CCl_4 to cattle was effective and safe, and that local tissue reactions were not serious if the dose was divided into four injections at different sites of the body. According to KOMJÁTHY (1957) a single s/c dose was not always completely effective in cattle, and WINTERHALTER & DELAK (1956) found this treatment to be ineffective against *F. hepatica* in cattle. Unsatisfactory results were also obtained by WINTERHALTER (1961) if a mixture of CCl_4 and oil was employed, to which hyaluronidase was added. It has been reported further, that treatment of cattle by s/c injection of CCl_4 causes necrosis of subcutaneous tissue at the site of injection, followed rapidly by a strong inflammatory reaction with abundant formation of connective tissue (WINTERHALTER & DELAK, 1956). KOMJÁTHY (1957) occasionally observed sterile abscesses at the sites of injection.

Several authors have reported favourably on the i/m use of CCl_4 in cattle: KOTLÁN & KOVÁCS (1957), HORVÁTH (1958), KOVÁCS (1959, 1959*a*), VESELOVA & VELIKOVSKAYA (1959), CERNI *et al.* (1960) and PEARSON & BORAY (1961). HORVÁTH (1958) employed a mixture consisting of equal volumes of CCl_4 and liquid paraffin, and administered 10 ml of this mixture per 100 kg body weight to 4,539 cattle. The efficacy of the treatment, determined by faecal examination, was stated to have been 94.2%. Injection of the mixture at the rate of 8 ml/100 kg seems to have yielded the same results. KOVÁCS also employed a mixture of CCl_4 and liquid paraffin in equal volumes, but a local anaesthetic (lidocaine) was dissolved in the mixture. The dose administered to about 150,000 cattle was either 8 ml of the mixture per 100 kg body weight with a maximum of 40 ml (KOVÁCS, 1959) or 10 ml mixture per 100 kg with a maximum of 50 ml per cow (KOVÁCS, 1959*a*). The dose was divided and injected in different sites. Both dose rates were claimed to have killed about 90% of adult flukes as determined by *post-mortem* examination two to four days after treatment, and to have completely cleared about 75–90% of the treated animals, as determined by faecal examination 14 to 21 days after medication. VESELOVA & VELIKOVSKAYA (1959) employed a mixture of CCl_4 and vaseline oil in equal quantities. It seems that a dose as high as 20 ml CCl_4 per 100 kg body weight has been administered to infested cows, and that no flukes were found at autopsy on the 5th and 7th day after treatment. In the livers of cows treated with half the dose, 10 to 28 flukes were found, whilst the livers of control animals yielded 23 to 115 flukes. The efficacy of a dose of 20 ml CCl_4 (per 100 kg body weight?), administered to 551 cows, was stated to have been 85%. CERNI *et al.* (1960) reported high efficacy in 93% of cattle 5 days after the i/m injection of 4 ml CCl_4 with 0.5% "Xilin" per 100 kg body weight. In an experiment carried out at Weybridge (MIN. of AGRIC., 1961), two calves received an i/m injection of 5 ml CCl_4 per 100 lbs body weight in 1/4 pint of liquid paraffin at the end of 22 weeks after the

administration of 1,000 encysted metacercariae. At autopsy, nine days after treatment, 3 and 4 flukes were found in the livers, whereas 64 and 141 flukes were found in two untreated controls. PEARSON & BORAY (1961) employed for i/m injection in cattle in Australia CCl_4 of either A.R. or commercial grade, and the drug was not mixed with any vehicle such as liquid paraffin. These authors concluded that CCl_4 at the rate of 1 ml per 20 lb body weight, with a maximum of 30 ml, was at least 90% efficient against mature flukes in about 90% of animals treated. This conclusion was based on the results of faecal examination in 90 cattle. One or two pre-treatment egg counts, and one to three post-treatment counts were carried out. The fact is significant that PEARSON & BORAY found living mature flukes in the livers of seven out of eight animals which were slaughtered after treatment (mean number of flukes less than four per liver), whereas no eggs had been found after medication in the faeces of four of these eight animals. In the light of these findings it would seem that the presence of small numbers of mature flukes, which survived treatment, has often not been detected by faecal examination, and that consequently the efficacy of the treatments has probably been overestimated.

In accordance with the results of investigations carried out by DELAK & ILIJAŠ (1961), WINTERHALTER (1961) reported that the absorption of a mixture of CCl_4 and liquid paraffin after i/m injection was enhanced by the addition of hyaluronidase to the mixture. The same appeared to be the case if a mixture of CCl_4 and sunflower-seed oil was employed. WINTERHALTER stated that i/m injection of CCl_4 mixed with either liquid paraffin or vegetable oil was ineffective against liver flukes in cattle if the mixture did not contain hyaluronidase. If the enzyme was added to the mixture the therapeutic results were considered to be comparable to or better than those obtained with hexachloroethane. WINTERHALTER found that the effectiveness depended on the proportion of CCl_4 and oil, and on the quantity of hyaluronidase. The more oil and hyaluronidase present in the mixture, the quicker and better was the action on the flukes. Increased amounts of oil, however, appeared to increase toxicity, for the author observed centrilobular necrosis of the liver parenchyma after i/m injection of a mixture of CCl_4 and vegetable oil in equal quantities together with 300 units of hyaluronidase, whereas this liver damage was not observed if the same amount of CCl_4 was injected together with smaller amounts of oil. The best results were obtained by WINTERHALTER with a mixture of two parts of CCl_4 and one part of vegetable oil and 150 units hyaluronidase. The therapeutic dose appeared to be 0.035 to 0.05 ml CCl_4 per kg of body weight. Eight animals were treated with this mixture and dose rate, and at autopsy two to four days after medication only dead flukes were found in five animals; in the livers of the remaining three animals, 5, 7 and 20 living flukes respectively were recovered together with dead flukes. WINTERHALTER used chemically pure CCl_4 for all his experiments.

Carbon tetrachloride appears to be remarkably well tolerated by cattle if injected intramuscularly .KOVÁCS (1959) experienced losses of 0.02–0.03% of 150,000 cattle

treated with doses up to 25 ml CCl_4 per animal. A few authors have observed clinical symptoms due to systemic toxic action of CCl_4 following i/m injection of the drug in cattle, but these symptoms do not seem to have been alarming. KOTLÁN & KOVÁCS (1957) ,who administered a dose of 6 ml/100 kg of a mixture of CCl_4 and liquid paraffin in equal volumes, observed moderate symptoms of excitation soon after injection in some animals, but these symptoms disappeared within 5–10 minutes; some other animals showed weakness and lack of appetite, and sometimes also diarrhoea two to three days after treatment. The daily milk production dropped slightly during a period of three to five days. By the addition of a local anaesthetic to the mixture employed for injection KOVÁCS (1959) appears to have been able to prevent excitement. Symptoms of dullness, decreased appetite, and inhibition of rumen contractions were noticed by KOVÁCS in 5–15% of the animals following treatment. VESELOVA & VELIKOVSKAYA (1959), who seem to have employed very high doses of CCl_4, observed various signs of intoxication in 20% of treated cattle: temperature rise by 0.5–1.5°C, acceleration of cardiac contractions, increased respiratory rate, and slight increase in the number of leucocytes and urobilin in the urine. Atonicity of the rumen was observed in 1–2% of medicated cattle. Following injection of a mixture of CCl_4 and vaseline oil in equal quantities at the dose rate of 4 ml/100 kg of CCl_4, DEMIDOV & VESELOVA (1959) observed that a small proportion of over 10,000 treated cattle became restless, ceased to ruminate, and were affected with muscular tremor. Symptoms lasted for 20 to 60 minutes. The animals were medicated in the spring, and they seemed to have received a diet deficient in mineral salts.

Local tissue reactions after i/m injection were considered by most authors to have been of minor importance if the dose was divided and injected at different sites. Severe lesions were observed at the sites of injection in the two calves treated at Weybridge (MIN. of AGRIC., 1961). WINTERHALTER (1961) also noticed that local tissue reactions in cattle as a result of i/m injection of a mixture of CCl_4 and either liquid paraffin or vegetable oil was severe. Necrosis and abundant formation of connective tissue was observed at the sites of injection. Addition of hyaluronidase to the mixtures was stated to have considerably reduced the severity of local tissue reactions. With the use of hyaluronidase the reaction consisted of mild muscular necrosis and extensive oedema in the intermuscular tissue, later followed by the formation of a relatively small amount of connective tissue. The mixture of CCl_4 and liquid paraffin caused much more severe tissue changes at the site of injection than did the mixture of CCl_4 and vegetable oil.

3. HEXACHLOROETHANE (C_2Cl_6)

A review of the literature on the history of C_2Cl_6 as a fasciolicide has been given by OLSEN (1947). This drug has almost entirely replaced CCl_4 for the treatment of

fascioliasis in cattle, and is occasionally used in sheep. The drug is administered orally, generally as a suspension in water.

Data on the efficacy of C_2Cl_6 against *F. hepatica* have been published by several authors, but only a few reports on critical tests are known to the writer. NÖLLER, FLIETNER & SCHMID (1928) treated cattle with 40 g C_2Cl_6 per 100 kg body weight, given in soft gelatin capsules. This dose was divided into four daily doses. Before treatment *Fasciola* eggs were found in the faeces of 20 animals, mostly in great numbers. Two months after the treatment only small numbers of eggs could be found in the faeces of 4 animals. A cow, which was slaughtered 17 days after treatment, appeared to harbour 124 normal living flukes. The bile ducts of this animal were thickened and heavily incrusted. The authors concluded that the drug was less effective when calcification of the bile ducts had already taken place. OLSEN (1947) used for medication of cattle in Texas an aqueous suspension, prepared by mixing 500 g of finely ground C_2Cl_6, 50 g of bentonite, 1/4 to 1/2 teaspoonful of white flour, and water sufficient to make 1,000 ml. The suspension was administered in doses of 200 ml (containing 100 g of C_2Cl_6) for grown animals, and 100 ml (containing 50 g of C_2Cl_6) for yearlings. Medication of 463 mature cattle, positive by faecal examination for liver flukes, resulted in only 8% of the animals showing eggs in their faeces when examined two to three weeks after the treatment. *Post-mortem* examination of medicated adult cattle, however, revealed that the efficacy of C_2Cl_6 against mature *F. hepatica* was considerably less than faecal examination before and after treatment seemed to suggest. In another experiment OLSEN medicated 91 adult cattle each with 200 ml of the mentioned suspension; hence the dose was 100 g of C_2Cl_6 per animal. These animals were slaughtered one to two weeks after treatment together with 52 untreated animals from the same pastures. The mature and immature flukes in the livers of the 143 animals were counted. Mature flukes were found to be present in 30% of the medicated, and in 73% of the unmedicated animals. In the case of immature flukes, the difference in the percentage of positive animals between the two groups was not regarded as being statistically significant. The mean number of mature flukes per animal was 1.4 for the medicated, and 20.8 for the unmedicated group. If it be assumed that the percentage of cattle infected with mature *F. hepatica* was the same in the medicated group before treatment as it was in the unmedicated group (i.e. 73%) it can be calculated that medication was completely effective against mature flukes in 59% of those of the treated animals which were originally infected with mature flukes.

These *post-mortem* examinations of adult cattle thus indicate that a high percentage of the mature flukes was destroyed by treatment with a single dose of 100 g of C_2Cl_6, but that a considerable percentage of the animals still harboured small numbers of flukes. The fact that faecal examination seemed to suggest a much higher efficacy than appeared from *post-mortem* examination is understandable, since ONO (1958) has found that liver flukes which survive C_2Cl_6 medication temporarily stop egg produc-

tion, and that ovulation recommences one month after administration of the drug. Faecal examination was performed by OLSEN two to three weeks after treatment.

OLSEN found that the greatest survival of flukes after medication occurred in livers or parts of livers which had undergone extensive pathological change due to fasciolíasis. This is in accordance with the experiences of ONO (1958), who medicated cattle with 30 g of a mixture of C_2Cl_6 and bentonite (10 : 2) per 100 kg body weight. ONO reported: .... "In the severely affected cases which have liver cirrhosis or advanced anaemia, the efficacy of the drugs is hopeless. For the effective treatment it is indispensable to diagnose the disease in early stage." The results obtained by OLSEN with calves support the view that a correlation exists between normal liver function and fasciolicidal activity of C_2Cl_6. Medication of fifteen 8-month-old calves, each with 50 g of C_2Cl_6, resulted in complete absence of living flukes in the livers one week later, while of fourteen comparable unmedicated calves twelve appeared to harbour from 1 to 20 living adult flukes in their livers. When pathological changes were present in the livers they consisted primarily of slight hypertrophy of that portion of the bile ducts which was occupied by the flukes.

Results similar to those of OLSEN have been obtained by EHRLICH *et al.* (1957) in Yugoslavia. These authors administered to each of 36 infected cattle of different age, sex, and state of health, 21 g of C_2Cl_6, 7 g of talcum, and 0.18 g of kaolin per 60 kg body weight on two successive mornings. When the animals were slaughtered (as far as the writer is able to make out 3 to 13 days after treatment) living adult flukes were found in the livers and gall-bladders of 19 animals. Thus medication was completely effective in about 47% of the animals. The average number of adult flukes, which remained alive in the 19 animals that were not freed of their infection, was 3.3 per liver. The authors state that young worms in the bile ducts were not killed by C_2Cl_6. Thirteen of the 19 animals remaining infected were affected with chronic lesions of the liver, with obstructed bile ducts. Surviving adult worms were never found in the lumen of unobstructed bile ducts.

The efficacy of C_2Cl_6 against *F. hepatica* in sheep has been determined by OLSEN (1948). These critical tests were part of a study in which the efficacy of C_2Cl_6 was compared with that of CCl_4. He administered to each of five sheep 30 ml of C_2Cl_6-bentonite suspension containing 15 g of C_2Cl_6. The sheep were slaughtered three to six days after medication. The living and dead flukes in the livers, as well as those which had passed with the faeces, were counted. The percentages of flukes destroyed in the five sheep by medication ranged from 68 to 100%, with the average of 91%. The treatment was completely effective in one sheep. Young flukes were not present. The results indicated that 15 g of C_2Cl_6 had the same effect as 1 ml of CCl_4 given orally.

By treatment of sheep at different intervals after administration of encysted metacercariae, DEMIDOV & POTEKHINA (1959) found that an oral dose of 0.3–0.4 g/kg of C_2Cl_6 did not result in death of immature flukes. When treatment was carried out

43 or 60 days after infection the drug appeared to be ineffective. Not until 11 and 12 weeks after infection, when sexually mature flukes were present, did the drug kill the flukes.

Although C_2Cl_6, which is virtually insoluble in water, is generally administered in the crystalline form, with or without inert additives such as bentonite, kaolin, talcum, or flour, solutions of the drug in organic vehicles have also been used. One of the first reports on the application of C_2Cl_6 against liver flukes was published by SCHMIDT (1926), who used a preparation, which was introduced on the market by a German manufacturer in that year under the name "Neoserapis". This fluid preparation was found by NÖLLER, GLUSCHKE & SCHMID (1927) to contain tetrachlorethylene (56%), C_2Cl_6 (23%), commercial benzene (containing toluene) (13%), and oil of turpentine (8%). It is known that tetrachlorethylene has no significant activity against *F. hepatica* in sheep (MONTGOMERIE, 1926*b*). HUPKA (1928) found "Neoserapis" to be completely effective against flukes in the bile ducts of cattle, and found that immature flukes, which were present in the liver parenchyma of one calf, had been killed.

Proprietary preparations which contain C_2Cl_6 in organic solvents, are still marketed. NICKEL & SOSSDORF (1953) used a preparation which contained C_2Cl_6, tetrachlorethylene, and liquid paraffin. MIMIOGLU & HOLZ (1955) reported that a preparation, which contained C_2Cl_6 and tetrachlorethylene, gave good results. Experiments in Australia appear to have shown that C_2Cl_6 dissolved in liquid paraffin or tetrachlorethylene is effective against *F. hepatica* in doses much lower than those which are required when powder preparations are used (ANONYMOUS, 1955). A report of a study on the comparative efficacy of both methods of using C_2Cl_6 is not known to the writer.

From most of the papers dealing with C_2Cl_6 as a fasciolicide it can be gathered that the drug is generally well tolerated by cattle and sheep, and that only in a small number of cases symptoms of intoxication, such as loss of appetite, diarrhoea, colic, dizziness, reeling and drowsiness occurred. These alarming symptoms generally passed quickly, but occasionally animals have died following treatment. The actual fatalities were estimated by HARROW (1959) to occur at the rate of one in every 20,000 cattle dosed and at about half this rate in sheep.

Contrary to these experiences are the facts recorded by a few authors. BYWATER (1955) reported that several cases of C_2Cl_6 poisoning of cattle have occurred in England. He stated: .... "In those cases brought to my notice, where deaths have occurred, there has been a herd susceptibility -all adult cattle dosed having exhibited nervous symptoms. Usually toxic symptoms and deaths have followed after the second part of the dose has been given, when only one-quarter or one-third of a conservatively computed therapeutic dose has been administered. All cases have occurred in animals on a low nutrition plane -usually grass plus some indifferent hay ...." Although it is impossible to compute the percentage of adult cattle which have died after administration of this drug it would appear that the death rate is in the region of 12 per

1,000 or more" .... "In those cases where I have reasonably accurate information, *post-mortem* examination has revealed either a low or normal fluke infestation without severe liver damage." .... BRANAGAN (1955) has communicated that intoxications as mentioned by BYWATER were successfully countered by administration of dextrose. The BRITISH VETERINARY CODEX (London, 1953) mentions a dose rate of 15 to 90 g of C_2Cl_6 for cattle, and HARROW (1959) stated: .... "The dosage generally recommended for cattle in Britain is now 15 g for every 6 months of age, with a maximum of 60 g for adult cattle". HARROW continues: "This, of course, applies to animals of normal growth and condition, and is reduced in the case of undersized or weak animals. In the latter case it is usually considered advisable to divide the dose into two parts, the second being given 48 hours after the first." According to HARROW Russian workers have recommended undivided doses as high as 240 g of C_2Cl_6 for cattle weighing 300 kg.

The tolerance of cattle towards this drug apparently varies extremely widely on different parts of the world. The highly productive dairy cows in Western Europe probably belong to the most sensitive animals, and doses as high as those used by OLSEN (1947) and by EHRLICH *et al.* (1957) are often likely to be poorly tolerated by cattle in this region.

Cases have also been recorded in which flocks of sheep have exhibited idiosyncrasy to C_2Cl_6. SOUTHCOTT (1951) medicated fifteen sheep each with 13.5 g of C_2Cl_6. Two sheep became ill, and one died. Another group of fifteen sheep was treated with 27 g of the drug per animal. After medication various degrees of intoxication occurred in eight sheep, two of which died. The sheep of both groups were in poor condition when they were treated. The drug was administered as a watery suspension of a proprietary C_2Cl_6-bentonite dispersible powder, which contained 90% C_2Cl_6. Fifteen comparable sheep, which received an oral dose of 1 ml CCl_4, developed no abnormalities. The symptoms of C_2Cl_6 intoxication appeared to be somewhat similar to those seen in hypocalcaemia. The author states that affected animals, in most cases, responded to injection of calcium borogluconate.

ENDREJAT (1953) reported that treatment of a flock of 200 sheep resulted in 40 deaths, whilst many animals were seriously ill for a long period.

The etiology of C_2Cl_6 poisoning has been discussed by HARROW (1959), who also described the symptoms and the *post-mortem* findings. He concludes: .... "to avoid accidents, feeding-stuffs other than grass or grass hay should evidently be withheld for several days before administration, and parturient or heavily lactating cows should not be dosed. It is advisable to test dose a few representative animals 48 hours before the main flock or herd."

Several workers have added to the C_2Cl_6 inert substances such as bentonite, kaolin, talcum and flour. These additives generally were used to facilitate the preparation of a suitable drench. A few authors believe that such additives, by their adsorbent properties, retard absorption of the active ingredient in the bowel, thus permitting

the use of large doses of C_2Cl_6 without causing serious intoxication. The writer has, however, found no evidence in the literature to support this view. Serious intoxications have also occurred when preparations were used which contained one or more of the mentioned additives.

The parenteral use of C_2Cl_6 has been tried by a few workers. NÖLLER & SCHMID (1927) found s/c injection of 10 g of C_2Cl_6 in olive oil in one sheep to be ineffective, but intraperitoneal injection of the same dose of C_2Cl_6 in liquid paraffin in another sheep appeared to be completely effective against the flukes in the bile ducts. In the latter case signs of intoxication were seen. VOGL (1954) considered that s/c injection of C_2Cl_6 in olive oil could be used for sheep, but SUGIURA (1960), like NÖLLER & SCHMID, reported s/c use to be ineffective. SUGIURA found that *F. hepatica* was killed by intraperitoneal injection of the drug when applied 60 days after infection of goats with the parasite, but that this treatment was ineffective if applied less than 40 days, or about 100 days after infection, when all the parasites were settled in the bile ducts and when cirrhosis and chronic cholangitis began to develop.

The writer is not aware of any parenteral use in the field of preparations of which C_2Cl_6 constitutes the only active ingredient.

4. COMBINATIONS OF FASCIOLICIDAL DRUGS

As has already been mentioned, preparations consisting of combinations of male fern extract and either CCl_4 or C_2Cl_6, have been used extensively in the past.

For the control of fascioliasis in sheep GRIGORYAN *et al.* (1955) used a combination of CCl_4 and C_2Cl_6. They reported that this treatment was much more effective than administration of either drug alone. LUNGU *et al.* (1959) employed a mixture of CCl_4, C_2Cl_6, and a mineral oil (probably liquid paraffin), which was injected subcutaneously in sheep. The treatment was stated to be effective. Higher doses were employed later, and a local anaesthetic was added to the mixture (LUNGU *et al.*, 1960). The authors claimed that all flukes died within 40 hours after injection of the mixture.

MITTERPÁK (1958) claimed that he has been able to destroy immature flukes in sheep by a combination of s/c injection of 1 ml of CCl_4 per 25 kg body weight and oral administration of 4 g C_2Cl_6 per 25 kg body weight. This remedy was successfully employed in 4,477 sheep.

C_2Cl_6 at 0.2 g/kg per os, together with 1 ml CCl_4, was administered by KHANBEGYAN (1960) to experimentally infected sheep and goats. Three to four days after this treatment no eggs were found in the faeces.

5. NEW FASCIOLICIDES

a. Efficiency

In efforts to find better remedies, numerous drugs have been tried against *F.*

hepatica in vitro and *in vivo* during the last decades. Reports on these screening tests have been reviewed by LÄMMLER (1955, 1956). A few new drugs which appear to possess activity have emerged from these tests.

DEMIDOV (1955) found that *symmetric difluorotetrachloroethane* ("*Freon 112*"), was effective if introduced into the stomach of sheep in doses of 0.3 to 0.6 ml per kg body weight, and did not produce clinically observable toxic effects. The same author reported later (1959) results obtained with 1,120 sheep. The drug, which was melted because of hot weather, was injected directly into the rumen in doses of 8–10 ml (0.2–0.3 g/kg). Fluke eggs were not found in the faeces ten days after medication. A few sheep showed various transient nervous symptoms. GNEDINA *et al.* (1958) stated that Freon 112, given intrarumenally in doses of 0.2 g/kg, reduced infection intensities by 66.7% in sheep but cured only one out of ten sheep. BORAY & PEARSON (1960) in Australia found that drenching of sheep with a 50% w/v mixture of difluorotetrachloroethane and liquid paraffin resulted regularly in a drop of 99–100% in faecal egg count, if a dose of 0.15 g of Freon 112 per lb body weight was administered. Living mature flukes were not found in the bile ducts of 29 treated sheep, which were previously infested, 19 days after medication. The treatment was well tolerated by all treated animals. Intrarumenal or intraabomasal injection of the mixture yielded similar results. The authors stated: . . . . "The effect on immature flukes was variable, and ranged from a complete kill to very little reduction in numbers. The low toxicity of "Freon 112" will permit higher dose-rates to be employed in future trials of its efficiency in acute fascioliasis." . . . . The mentioned experiments were described in detail in another publication (BORAY & PEARSON, 1960*a*).

DEMIDOV (1959*a*) tested Freon 112, with small additions of Freon 12 (difluorodichloromethane) to lower its melting point, against fascioliasis in cattle. Doses of 0.3 and 0.4 ml/kg, administered by mouth or directly into the rumen by injection, were found to have cured 80% and 100% of animals respectively in a first experiment where autopsy was performed four days after treatment, and 71.6% and 86% in a second experiment where autopsy was made after eight days. The mixture was harmless for cattle at these dose rates.

Further discoveries in the field of chemotherapy of fascioliasis will be treated in chronological sequence, because, apart from the following report by HIRSCHLER, all further publications appeared after a preliminary communication by the writer (DORSMAN 1959) about some results of treatments of cattle with hexachlorophene.

One of the many drugs, which were tested by HIRSCHLER (1957) on liver fluke infected sheep, was *2,2'-methylenebis (3, 4, 6-trichlorophenol)*, which is known as *hexachlorophene* or as *G-11* (R). A solution of the drug in dehydrated alcohol was injected subcutaneously in one sheep at the dose rate of 200 mg hexachlorophene per

(R) Registered trade-mark, Givaudan-Delawanna, Inc.

kg body weight. Five days after the treatment eggs of *F. hepatica* were no longer found in the faeces, and when the animal was slaughtered on the 9th day after medication, no *F. hepatica* could be found in the bile ducts.

After having taken note of HIRSCHLER's results the writer tried hexachlorophene at different dose rates against *F. hepatica* in cattle. The drug was administered either orally (suspended in water or dissolved in olive oil), or subcutaneously (dissolved in dehydrated alcohol or in olive oil). Some results were published in a preliminary communication (DORSMAN, 1959).

Soon afterwards LIENERT (1959) reported that *F. hepatica*, which had been implanted in the abdominal cavity of rats, were killed by administration of hexachlorophene to the host, and that the drug had also been found to be effective against the same parasite in sheep.

At the same time the writer presented a second preliminary note (DORSMAN, 1959*a*) on results obtained with hexachlorophene and some related compounds against *F. hepatica* in cattle.

The writers experience that hexachlorophene has outstanding activity against *F. hepatica* in cattle was confirmed by FEDERMANN (1959), who also reported results obtained with this drug against liver fluke in sheep. FEDERMANN treated four infected cows with 10 mg G-11 per kg body weight, and another four with 20 mg per kg. The drug was administered orally as a watery suspension. After the animals were slaughtered eight days later, no living flukes could be found in the livers of three animals which had received 10 mg G-11 per kg, or in the four animals which had been treated with the higher dose. *Post-mortem* examination of twenty sheep, which had each received orally a watery suspension containing 20 mg G-11 per kg body weight eight days earlier, showed that the treatment was completely effective. A dose of 15 mg G-11 per kg was found to have freed 19 out of 20 sheep of their infection, while a dose of 10 mg G-11 per kg appeared to have killed all the flukes in the bile ducts in 11 out of 20 sheep. Five untreated control animals appeared to harbour an average of 68 flukes per liver. Faecal examination of groups of infected sheep suggested that subcutaneous injection of an oily solution of the drug at a dose rate of 15 mg per kg was somewhat less effective than oral administration of the same dose of the drug suspended in water.

Hexachlorophene was found in Japan to be effective against *Fasciola* in rabbits (UENO *et al.*, 1959) and in sheep (UENO *et al.*, 1960*a*). The latter animals were almost completely freed from flukes by a dose of 10 mg/kg, as determined by *post-mortem* examination.

Results of treatment of liver fluke infected cattle and sheep in the Netherlands with hexachlorophene were recorded by OSINGA (1960). Activity against liver fluke in cattle was estimated by faecal examination about four weeks following medication, and by clinical improvement as compared with that obtained after oral administration of C_2Cl_6 (12.5 g per 100 kg body weight on each of two subsequent days, with a

maximum of 50 g). Clinical results of treatment with G-11 against fascioliasis in sheep were compared with those of treatments with CCl_4 (1 ml on each of two subsequent days) of the same sheep in the foregoing months. The treated cattle and sheep were stated to have all been suffering from clinical fascioliasis. Nine cattle received a subcutaneous injection of a 10% solution of hexachlorophene in olive oil at a dose rate of 10 to 15 mg G-11 per kg body weight.* About four weeks later *Fasciola* eggs were found in the faeces of eight out of the nine animals. For oral administration of hexachlorophene to cattle 10% solutions of the drug in either polyethylene-300 (probably polyethylene glycol 300) or propylene glycol were employed. A drench was prepared for each individual animal by mixing the required quantity of G-11 solution with about 500 ml water. In this way 51 cattle were medicated at a dose rate of 10 to 12.5 mg G-11 per kg body weight. *Fasciola* eggs were found in the faeces of 67% of the animals about four weeks after medication, whereas the faeces of all twenty comparable animals, which had been treated with C_2Cl_6, appeared to contain *Fasciola* eggs about four weeks after treatment. For the treatment of 23 sheep the 10% solution of G-11 in propylene glycol was used to prepare a drench for each individual animal. The dose administered was 10 to 12.5 mg G-11 per kg body weight. Faecal examination was not performed. After the treatment 21 out of the 23 sheep showed marked clinical improvement, whereas no beneficial effect had been observed in the foregoing months after repeated treatments of the same animals with carbon tetrachloride. The two sheep, which did not improve in condition after the treatment with G-11, died some weeks later. Flukes could not be found in the badly damaged livers of both animals. OSINGA concluded that hexachlorophene did not give significantly better clinical results in the treatment of fascioliasis in cattle than did hexachloroethane, whereas clinical results of medication with G-11 in sheep were better than those obtained with carbon tetrachloride. G-11 was found to be more toxic than C_2Cl_6 at the employed doses.

STREHL (1960) treated four sheep, which were positive for liver flukes by faecal examination, by subcutaneous injection of 15 mg/kg of hexachlorophene. The drug was dissolved in N:N-dimethyl-acetamide, which was emulsified in distilled water. When the animals were slaughtered 5 to 39 days after treatment, no living flukes could be found in the livers.

Three infected cows were also treated by injection: two animals received a subcutaneous injection of a similar emulsion as used for sheep, and in the third animal a solution of hexachlorophene in Tween 80 was injected intramuscularly. The dose for each of the animals was 15 mg/kg of G-11. The cow which recieved an intramuscular injection was slaughtered about three weeks after injection. No flukes were found in the liver. The two other animals were slaughtered somewhat more than two months after treatment. In the liver of one of these cows no flukes were found, but the liver

* The body weights of all experimental cattle and sheep were estimated.

of the other animal yielded eight living flukes. As more than two months elapsed between treatment and slaughter, the possibility should be considered that at least some of these flukes were immature when the host was treated.

In a preliminary communication NEMESÉRI (1960) reported results obtained in Hungary by administration of hexachlorophene to sheep and cattle which were suffering from fascioliasis. In one experiment 182 sheep and 156 cattle received a dose of 15 to 20 mg/kg of G-11 by mouth, and another ten cattle were given 15 mg/kg of G-11 by injection into the rumen. In this experiment hexachlorophene was administered as a suspension of one part of the drug in ten parts of liquid paraffin. About 90% of the animals were stated to have been completely freed of infection at the dose rate of 15 mg/kg of G-11, and over 91% at the higher dose rate.

GUILHON & GRABER (1961) treated sheep with hexachlorophene by administering the drug in gelatin capsules by mouth, or by subcutaneous injection of a 15% solution of G-11 in olive oil. Four of these animals were carrying a light infection of *F. hepatica*. Living specimens of *F. hepatica* were found in the liver of a sheep which was slaughtered on the 4th day after subcutaneous administration of 20 mg/kg of G-11, and also in the liver of another sheep slaughtered on the 9th day following administration of 30 mg/kg of G-11 by the oral route. No living flukes were found in the livers of the two remaining sheep which were previously infected with *F. hepatica*, and which had received G-11 by the oral route. One of these animals was slaughtered the 2nd day after the administration of 30 mg/kg of G-11; the details of the other animal are not clear. Sheep infected with *Fasciola gigantica* were completely cured by an oral dose of 10 to 20 mg/kg of G-11.

UENO *et al.* (1959) reported from Japan on the fasciolicidal action of two compounds which are chemically related to hexachlorophene: *2,2'-thiobis (4,6-dichlorophenol)*, also known as *bithionol*, and *bithionol acetate*. Both drugs were found active against liver fluke in rabbits, and the former also against the parasites in sheep. For effective treatment of sheep a dose of at least 50 mg/kg of bithionol was required. Young liver flukes were not killed in rabbits by bithionol at a dose rate of 100 or 200 mg/kg or in sheep at the dose rate of 75 mg/kg. A dose of 75 mg/kg of bithionol was later reported by the same authors (UENO *et al.*, 1960*a*) to be nearly completely effective against *Fasciola* (apparently mature) in sheep, while a dose of 35 or 50 mg/kg had no effect.

Excellent results have also been obtained against liver flukes in cattle by administration of bithionol by the oral route (UENO *et al.*, 1960). Single doses of 35 to 55 mg/kg were found to be fully effective against mature flukes in six cows, and a dose of 30 mg/kg freed one of two cows from all mature flukes. A dose of 20 mg/kg of bithionol, administered to five cows, resulted in death of about 50% of the flukes.

WATANABE (1958) stated that liver flukes, which could be identified as *Fasciola hepatica* have not often been seen in Japan. The common liver fluke in that country was considered to be either *Fasciola gigantica* or an intermediate form of *F. gigantica*

and *F. hepatica*. This statement is very important with respect to the results obtained by UENO *et al.* with bithionol against liver flukes, for indications can be found in the literature that the effect of drugs on *F. gigantica* differs from that of *F. hepatica*.

Another new drug was introduced by LÄMMLER (1960): *1,4-di(trichloromethyl) benzene*. The drug is marketed as a fasciolicide under the name "Hetol", as a wettable powder, which contains 85% of the active ingredient. 1,4-Di(trichloromethyl) benzene is practically tasteless and odourless, and it is insoluble in water. LÄMMLER tested the effect of a single oral dose of the drug in watery suspension against *F. hepatica* in rabbits and rats which had been experimentally infected, and in sheep and cattle with naturally acquired infection.

Rats dosed with at least 60 mg/kg of the drug were found by *post-mortem* examination a week after treatment to have been completely freed of infection. Doses lower than 55 mg/kg were ineffective. A dose of 500 mg/kg, administered to an infected rabbit, resulted in complete necrosis of all flukes which were present in the bile ducts three days after treatment.

The efficacy of the drug against liver flukes in sheep and cattle was mainly determined by egg counts in faecal samples which were collected at intervals of one week during a period of at least four weeks. *Post-mortem* examination after treatment was performed on a few animals.

Trials with increasing doses of the drug, administered to sheep, had the following results: no eggs of *F. hepatica* were found 2, 3, or 4 weeks after treatment if the dose was 150 mg/kg or higher; lower doses yielded variable results. It must be remarked that the pre-treatment egg counts were very low: these ranged from 8 to 80 eggs per gram of faeces in the 15 experimental sheep. Three sheep, which had been treated with 150 mg/kg of 1,4-di(trichloromethyl)benzene, were slaughtered 3 to 20 days after medication. No living flukes could be found in the bile ducts which had undergone advanced pathological change.

In another experiment LÄMMLER (1960*a*) administered 150 mg/kg of 1,4-di(trichloromethyl)benzene to fourteen sheep. Faecal egg counts before treatment were very low: numbers of less than 100 eggs per 10 g faeces were recorded in 11 animals, and no eggs were found per 10 g faeces in two of these. More than 100 eggs per 10 g faeces were counted in faecal samples of three animals. Two weeks after medication no eggs were found per 10 g faeces in 13 animals, and 21–50 eggs per 10 g faeces in the remaining animal. Four weeks following treatment the number of eggs per 10 g faeces was found to be less than one in 11 sheep, 1–20 in two animals, and 51–100 in one sheep.

The curative dose for cattle was determined by LÄMMLER (1960) in the same way as for sheep. The faecal examinations suggested that a dose of 125 to 135 mg of the drug per kg body weight was sufficient to kill all mature flukes. The pre-treatment egg counts of the 15 experimental cattle were also very low: these ranged from 0.2 to

7 eggs per gram of faeces. The body weights indicate that the majority of those 15 cattle were young animals. Five cattle were slaughtered 3 to 20 days after having received a dose of 125 to 135 mg of the pure drug per kg body weight, and no flukes were found in the livers which were severely affected as a result of fascioliasis.

BEHRENS (1960) determined the effectiveness of "Hetol" in the treatment of fascioliasis in sheep by faecal examination. Thirteen flocks, comprising 3,383 animals, were medicated with a watery suspension which contained 20% "Hetol". Lambs aged 5 months and older received 25 ml (5 g "Hetol"), and sheep more than a year old 50 ml (10 g "Hetol"). The average weight of the latter was estimated to be 50 kg. Before treatment faecal samples were collected from at least ten animals from each flock. Four and eights weeks following treatment faecal samples were examined from ten to twenty sheep per flock. Six of the flocks had been housed for one to two months before they were treated. According to faecal examinations carried out before treatment the level of infection in three flocks had been 100%, and had varied between 30% and 90% in each of the other three. Faecal examinations, carried out four weeks after treatment, showed the level of infection in all six flocks to vary between 0% and 10%, and eight weeks after treatment between 0% and 5% in five of the six flocks. The results with flocks of grazing sheep were more variable. Two of these latter had been grazing on pastures which were known to be heavily contaminated with encysted metacercariae of *F. hepatica* for some weeks before treatment. Consequently these sheep could be expected to harbour many immature flukes on the day of treatment. Eight weeks after treatment the levels of flock infection with mature flukes were found to be 20% and 25% respectively. After the same period following treatment the levels of infection in the other five grazing flocks, however, were found to vary between 0% and 10%. These results suggest that many flukes, which were immature at the moment of treatment, were not affected by the drug and had grown mature afterwards.

The effect of "Hetol" at the mentioned dose rate was also compared by BEHRENS with that of hexachloroethane (8 grams per sheep, suspended in water), and with C_2Cl_6 in the form of pills (dose not mentioned), by treating comparable groups of twenty infected sheep. Four weeks after medication 75% of the animals of the "Hetol"-group were found negative for liver flukes as indicated by faecal examination, whereas no eggs were found in 35% of the animals of both C_2Cl_6-groups. The drop in faecal egg count in the three groups was 96.4%, 79% and 75% respectively. "Hetol" thus seemed to be much more effective than the hexachloroethane preparations.

The activity of 1,4-di(trichloromethyl)benzene against *F. hepatica* in cattle was confirmed by ENIGK & DÜWEL (1960). They treated 864 animals of different ages with naturally acquired infection, with 8 grams of "Hetol" per 50 kg body weight (*i.e.* 136 mg of 1,4-di(trichloromethyl)benzene per kg)* with a maximum of 80 g

* 187 Cattle could be weighed; the body weights of the others were estimated and rounded off upwards to 50 kg for dosage.

"Hetol". About 40% of the animals were heavier than 500 kg, so that these received less than 8 g "Hetol" per 50 kg. The efficacy of the drug was determined by repeated faecal examinations and by *post-mortem* examination of 49 animals 7 to 51 days after treatment. In spring (January to March) 581 infected animals were treated. The animals were considered to harbour only mature flukes at that time of the year. Repeated examination of faecal samples up to eight weeks after treatment showed that 75% of the animals had stopped excreting eggs with the faeces, while the number of eggs was considerably reduced in the faeces of the other animals. Twenty animals out of the group of 581 were slaughtered 7 to 20 days after medication. No flukes were found in the livers and gall-bladders of 18 of the animals, and in the organs of the two others 2 and 4 living or affected flukes were found respectively. The results with 283 cattle, which were treated in autumn (September to November), were not so good. At that season the animals have been continuously reinfecting themselves, and immature flukes had apparently escaped the action of the drug.

It appeared that in cases where fascioliasis had given rise to marked liver cirrhosis and incrustation of the bile ducts the drug was less effective than in cases of recent infection.

ENIGK & DÜWEL are of opinion that the chemotherapy of fascioliasis in cattle has been improved by the introduction of "Hetol", but that control of the intermediate snail host has not become superfluous. Apart from the presence of infected game animals, which contaminate the pastures with *Fasciola* eggs, infection of the snails continues because the excretion of fluke eggs with the faeces is not completely stopped in all cattle by treatment with this new drug. Consequently reinfection of farm animals cannot be prevented by repeated treatments.

EHRLICH *et al.* (1960) presented evidence that acute fascioliasis due to migrating young flukes could be successfully treated with *Mepacrine hydrochloride (Atebrine)*. *Post-mortem* findings and clinical observations in cattle after intravenous administration of the drug at the dose rate of 15 mg/kg, once, or twice within three to five days, indicated that immature flukes in the liver parenchyma were killed. Young flukes present in peritoneal exudate were not affected by the treatment.

EUZÉBY & BUSSIÉRAS (1960) reported results obtained in sheep and a calf with *diethylcarbamazine*, and in sheep with *4,7-phenanthroline-5,6-quinone* (Ciba 11'925c, "Entobex"). The authors stated that both drugs increased the excretion of ova by *F. hepatica*.

b. Toxicity

Symmetric difluorotetrachloroethane (Freon 112)

GREENBERG & LESTER (1950) administered the drug to adult white rats in a dose of 2 g/kg/day for periods of 23 to 33 days. There was no pathological change in any organ.

No clinical symptoms of toxicity were observed by DEMIDOV (1955) and by BORAY & PEARSON (1960, 1960*a*) after administration of Freon 112 at therapeutic dosage to sheep, but DEMIDOV (1959) observed transient nervous symptoms in a few sheep when the treatment was apparently applied on a larger scale. The symptoms were more pronounced in sheep which were starved for 20 hours before treatment than in those which were not starved. BORAY and PEARSON (1960*a*) administered a dose of 0.75 g/lb of Freon 112 to a sheep by mouth; another received the same dose by intra-abomasal injection, and a third sheep was given an oral dose of 1.0 g/lb body weight. No symptoms of toxicity were observed, and no pathological changes were found in the liver, kidney and myocardium after the animals had been slaughtered 24 hours following treatment.

DEMIDOV (1959*a*) stated that the mixture of Freon 112 and Freon 12 at the dose rate of 0.3 or 0.4 ml/kg was harmless for cattle, and that the maximum non-lethal dose was 3 ml/kg.

Hexachlorophene (G-11)

The SINDAR CORPORATION (1955) has published information on the toxicology of hexachlorophene: "Data on the acute oral toxicity of G-11 vary somewhat. For mice, NICKERSON found a LD_{50} of 187 mg/kg with a range of 166 to 209 mg/kg, whereas FLORESTANO (1949) reported a LD_{50} of 80 mg/kg. Tests on guinea pigs showed a LD_{80} of 280 to 300 mg/kg. A sub-acute toxicity study for a thirty-day period indicated that G-11 is mildly toxic when fed to rats in a concentration of 1 to 5000 and toxic to a greater degree when fed in a concentration of 1 to 2500." . . . .

Hexachlorophene, administered orally, was found by UENO *et al.* (1959) to be very toxic to rabbits at the dose rate of 100 mg/kg, and by the same authors (1960*a*) to cause side effects in sheep at the dose rate of 20 mg/kg.

KERR (1948), who discovered the anthelmintic activity of hexachlorophene against poultry tapeworms, conducted toxicity studies of this drug in chickens. He stated: . . . . "With regards to the lethal dose, 500 mg per kg body weight is lethal, most of the birds dying within 24 hours after medication. The outward sign of acute toxicity is an increased respiratory rate which is sometimes accompanied by a rise in body temperature. Autopsy shows engorgement of the smaller superficial blood vessels, the right auricle, the liver, and frequently the kidneys. Birds dying a less acute death apparently succumb to a nephritic condition. One of four birds given a dose of 400 mg per kg body weight died of acute toxicity. The other three birds survived, showing a loss of weight for 7 days following medication. None of the four birds given 300 mg per kg body weight died. These birds showed no toxic signs other than a temporarily lessened rate of weight gain. Eleven birds were given a dose of 200 mg per kg body weight and four of these birds died. Three of the survivors showed a temporary loss in weight, but the remaining four birds exhibited no toxic reaction. Occasional small temporary weight losses occur at dosages of less than 200 mg per kg body weight." . . .

ENIGK & DÜWEL (1959) observed that chickens kept separately in batteries tolerated 150 mg hexachlorophene per animal, whereas birds on free range sometimes died following the same dose.

FEDERMANN (1959) performed toxicity tests with rats, sheep, and cattle. Rats weighing 170 g were given hexachlorophene orally as a 30% wettable powder at the dosages of 100, 250 and 500 mg per kg body weight. All ten rats dosed with 100 mg per kg survived; at the dose of 250 mg per kg four out of the ten survived, and all ten rats which had received 500 mg per kg died.

Forty sheep were each treated with two doses of 40 mg G-11 per kg body weight, administered on two subsequent days. The treatment was repeated three weeks later. All forty animals appeared to be normal during the entire period of the experiment. A single dose of 60 mg G-11 per kg body weight, administered to twenty sheep, resulted in death of four animals, while two sheep showed marked paralysis of the fore- and hind-quarters during the first day following administration of the drug. Subcutaneous injection of 1 ml of a 15% solution of hexachlorophene in oil per 10 kg body weight, gave rise to slight to considerable swelling at the site of injection 12 to 24 hours later in 100 sheep treated in that manner.

FEDERMANN has treated a total of 1011 sheep of various ages and breeds with doses of 10 to 20 mg G-11 per kg. Apart from the local tissue reactions in the sheep which were injected subcutaneously, no intolerance towards the drug was observed.

Oral medication of fifteen cattle at a dose rate of 40 mg G-11 per kg body weight resulted in death of one animal five days after treatment. The other fourteen animals did not show any abnormality. No signs of intoxication were observed in ten cattle which had received a dose of 20 mg G-11 per kg orally.

OSINGA (1960) reported that subcutaneous injection of a 10% solution of hexachlorophene in olive oil in nine cattle resulted in big and painful swellings at the site of injection, which disappeared only after some months. The applied dose was 10 to 15 mg G-11 per kg body weight. Symptoms of systemic intoxication were not observed. For oral administration of hexachlorophene to cattle solutions of the drug in organic solvents were employed, as already described. After having treated 51 cattle in this way at a dosage of 10 to 12.5 mg/kg of G-11, four animals showed signs of indigestion with diarrhoea, and decreased milk yield. One of these animals, a two-year-old cow, which had calved four weeks earlier, developed grass tetany-like symptoms, which became so alarming that the animal had to be slaughtered. A report on *post-mortem* examination has not been given. The condition of the animal before treatment was stated as having been fairly good. Several of the 23 sheep suffering from clinical fascioliasis, which OSINGA had given an oral dose of 10 to 12.5 mg/kg of G-11, were dull, showed lack of appetite and sometimes had diarrhoea the first two days following medication. For the treatment of sheep a 10% solution of hexachlorophene in propylene glycol was employed. As organic solvents have been used by OSINGA as vehicles for hexachlorophene, their toxicity should also be considered. JONES (1959)

stated that propylene glycol is relatively non-toxic, and that it is a normal intermediate in the formation of glycogen from acetone. He recommended propylene glycol for the treatment of bovine ketosis at the dose of 500 g, administered orally in two divided doses for four to five days. These are doses far in excess of those applied by OSINGA. The acute oral toxicity of polyethylene glycol 300 is also very low (SMYTH *et al.*, 1950). Although the solvents thus can be considered as completely harmless in the doses employed by OSINGA, the possibility can not be excluded that their presence has enhanced the absorption of hexachlorophene, and thus has increased the acute toxicity of the latter.

The tolerance of parenterally administered hexachlorophene in sheep and cattle has been investigated by STREHL (1960). Seven sheep received a subcutaneous injection of an emulsion prepared from hexachlorophene, N:N-dimethyl-acetamide, an unspecified emulsifier, and distilled water. Six of the sheep received 15 mg/kg of G-11, and the remaining animal 30 mg/kg. A local tissue reaction was observed in one of the animals which had received 15 mg/kg of G-11. None of the animals showed signs of intoxication.

All eight cattle, which were injected subcutaneously either with a similar emulsion as employed for sheep or with a solution of hexachlorophene in polyethylene glycol monoethylether 400, developed local lesions. The dose rate was 15 mg/kg of G-11. General toxic reactions were not observed. One animal, which weighed 350 kg, received an intramuscular injection of a solution of 5 g hexachlorophene in 10 ml Tween 80. During the day following treatment the animal was dull and did not eat. A painful local lesion developed during the week following injection. The chemicals which have been used by STREHL in combination with hexachlorophene appeared not to cause tissue reactions in sheep and cattle if injected subcutaneously without G-11.

STREHL found that the blood picture of the experimental sheep and cattle was not changed after medication. The treatments also appeared to have not influenced the serum calcium, potassium and sodium levels. Bilirubin and protein never appeared in the urine of the sheep and cattle after medication, and the pH of the urine remained within the normal limits.

NEMESÉRI (1960) administered hexachlorophene at the dose rate of 15 mg/kg by subcutaneous or intramuscular injection. The drug was either dissolved in alcohol (154 sheep and 10 cattle) or suspended in ten parts of liquid paraffin (321 sheep and 16 cattle). Side effects were observed in the form of a slightly limping gait for up to two weeks in sheep, and excitation during two to three hours in cattle. No side effects were observed in 182 sheep and 156 cattle which had received 15–20 mg/kg of G-11 by the oral route, or in another 10 cattle which had received 15 mg/kg by injection into the rumen. In these cases the mentioned suspension of G-11 in liquid paraffin was used.

GUILHON & GRABER (1961) observed a remarkable difference in sensitiveness towards hexachlorophene when they treated sheep at Alfort (France) and at Fort-Lamy (Tchad, Africa). Sheep of very different age, body weight, and condition, were

treated in France, generally by oral administration of G-11 in capsules, a few by subcutaneous injection of a 15% solution of the drug in olive oil. These animals tolerated doses up to 60 mg/kg of G-11 without showing signs of intoxication. One animal, which received 30 mg/kg of G-11 by intramuscular injection of the oily solution was affected by paralysis after treatment. Following a dose as high as 100 mg/kg of G-11, administered either per os (3 animals) or subcutaneously as the 15% oily solution (1 animal), all four animals exhibited severe symptoms of intoxication, and three sheep (including the animal that was treated by subcutaneous injection) died. One lamb, weighing 15 kg, received three treatments with hexachlorophene: 30 mg/kg per os, 60 mg/kg and 100 mg/kg by subcutaneous injection, at intervals of ten and seven days respectively. This animal showed only slight prostration and increased respiratory rate for about 24 hours after the third treatment (100 mg/kg). Whilst the French sheep generally tolerated up to 60 mg/kg of hexachlorophene, three out of four African sheep died two to four days after administration of 40 mg/kg of the drug by the oral route. The one animal that survived did not show any apparent reaction. The following symptoms, which developed earlier after treatment and were more pronounced when the administered dose was higher, were observed by GUILHON & GRABER in intoxicated sheep: loss of appetite, weakness, immobility or moving with uncertain steps, staggering gait when forced to move, a stance with spread limbs and an unsteady equilibrium, and finally, complete paralysis preceding death. The head was often either kept low or just raised. The pupils were often considerably dilated (amaurosis), and the ears showed a slight trembling. Increased respiratory rate was also observed. The authors reported that cattle reacted more irregularly than sheep to hexachlorophene, and were more prone to toxic effects. Several cases of intoxication were observed in cattle after administration of 20 to 40 mg/kg, and fatalities occurred after doses of 20 to 25 mg/kg and higher. Hexachlorophene intoxication in chickens has also been studied by GUILHON & GRABER (1961*a*). The minimum lethal dose was found to be 80–100 mg/kg. Intoxicated birds showed loss of appetite. They assumed a recumbent position with drooping wings and the beak wide open; their noisy breathing was accompanied by an increased respiratory rate. Autopsy revealed pulmonary oedema, a slight congestion in the intestine and the liver, and sometimes nephritis.

KOK (1961) reported investigations on the fate of hexachlorophene in rats and mice. The results suggested that the drug is slowly and incompletely absorbed in the rat after oral administration, and that it is metabolized to a marked degree. No evidence was found for a specific affinity of G-11 for liver, muscle, brain, lung, kidney and fat. The presence of the drug in muscle, brain, and fat could not even be demonstrated. Urinary excretion of unchanged hexachlorophene was found to be nil.

Bithionol

According to the MONSANTO CHEMICAL COMPANY (1955) the acute oral toxicity of

"Actamer" (a brand of bithionol, U.S.P.) in several media has been determined in three independent laboratories for rats, mice, guinea pigs, and rabbits. If a watery suspension of the drug was administered, the acute oral toxicity (LD_{50}) was stated to be for rats 5.77 g per kg, for mice 1.428 g per kg, and for rabbits 2.1–4.7 g per kg. Suspensions of bithionol in vegetable oils appeared to be more toxic. Dogs given doses of 3.2, 4.7, and 7.0 g of "Actamer" (as a 20% suspension in 1.5% aqueous methyl cellulose) per kg body weight survived, showing no signs of illness and displaying normal appetites and activity.

UENO *et al.* (1959) reported that rabbits and sheep given orally a dose of 100 mg/kg of bithionol did not show any side reaction. Doses of 100 mg/kg and higher were later reported to have caused side effects in sheep (UENO *et al.*, 1960*a*).

The only indications of toxicity observed by ENZIE & COLGLAZIER (1960) were emesis in dogs and a softening of the faeces in dogs, cats, chickens and a lamb after administration of the following dosages of bithionol: dogs 50–250 mg/lb, cats 50–250 mg/lb, chickens 100–500 mg/lb, and a lamb 100 mg/lb.

1,4-Di(trichloromethyl)benzene

LÄMMLER (1960) studied the acute and chronic toxicity of 1,4-di(trichloromethyl) benzene. The maximum tolerated single oral dose for mice, rats, and rabbits appeared to be >4000, 2800, and 2200 mg/kg respectively. A single oral dose of 600 mg per kg body weight was well tolerated by sheep. Oral administration to sheep of a single dose of 750 to 1500 mg/kg resulted only in diminished appetite, some loss of vitality, or apathy, during the first one or two days following dosing. A single oral dose of 150, 180, 200, 250, 260, 300, and 390 mg/kg respectively was administered to each of seven cattle. Two animals (150 and 250 mg/kg) did not show any reaction, and the remaining animals exhibited no other symptoms apart from some loss of appetite and vitality during 12 to 24 hours. Chronic toxicity studies, performed by LÄMMLER, had the following results. A daily oral dose of 800 mg/kg, administered to five rats during 15 subsequent days, led to the death of two animals. All rats dosed daily with 400 mg/kg or less during 15 days survived. Pathological tissue changes were observed in rats which received 15 daily doses of 400 or 800 mg/kg, but not in those dosed daily with 50 to 200 mg/kg. The only symptoms exhibited by a sheep that received twenty daily oral doses of 100 mg/kg was a slightly diminished uptake of food, which resulted in a weight loss of 12%.

The chemotherapeutic index for 1,4-di(trichloromethyl)benzene was found to be >10 in sheep, and >3 in cattle. As a result of his investigations LÄMMLER recommends a dose of 150 mg/kg for sheep, and of 135 mg/kg with a maximum of 68 grams (80 g "Hetol") for cattle.

Medication with "Hetol" was carried out by LÄMMLER in 880 sheep and 55 cattle in the field. Five to ten percent of the sheep treated in May and June showed a diminished food intake, which disappeared within twelve hours, on the second day

following medication. Other signs of intolerance were not observed in the sheep, and the cattle did not show side effects.

BEHRENS (1960) observed that a therapeutic dose of "Hetol" is not always tolerated by sheep which are fed sugar-beet and beet leaves. Four sheep died, 18 to 28 hours after medication, in a flock of 450 animals on a beet diet. The sheep that survived did not show signs of intoxication. The sensitivity of another flock, which was fed in the same way, was tested by treating ten sheep, with the result that one sheep died. When sugar-beet was withheld the day before, and three days following medication, no intoxication was observed in the remaining 344 sheep of that flock. The same precaution was also completely successful in two other flocks which had been fed fresh beet. Moreover, treatment was perfectly tolerated by nine other flocks (a total of 2,091 animals) which did not receive fresh beet.

ENIGK & DÜWEL (1960) found that 12 g "Hetol" per 50 kg body weight (204 mg/kg of 1,4-di(trichloromethyl)benzene), which is 50% more than the therapeutic dose as proposed by LÄMMLER, did not cause signs of intoxication in 23 young cattle. The normal therapeutic dose was well tolerated by housed and grazing cattle, even by sick or pregnant animals, and by cows in early lactation. The administration of different kinds of concentrated food did not produce sensitivity of the animals to the drug. However, the provision of fresh beet, beet leaves, and cabbage has led to various degrees of transitory intoxication in about 60 to 70% of the treated cattle which were given these foods. Rather serious symptoms (loss of appetite, colic, cessation of milk production) were observed in about 15% of these animals. All patients recovered completely within a week.

EIKMEIER & KAMEL (1961) studied the tolerance of sheep towards a dose of 150 mg/kg of 1,4-di(trichloromethyl)benzene by means of laboratory methods and clinical observation. They concluded that no indications could be found for toxic reactions. The animals received a diet of hay or of a mixture of hay and fresh grass from two to three days before treatment.

Mepacrine hydrochloride

EHRLICH *et al.* (1960) observed that the therapeutic dose of 15 mg/kg usually resulted in more or less pronounced symptoms of short duration due to inhibition of acetylcholine-esterase activity. Doses of 25 mg/kg of Mepacrine hydrochloride were well tolerated by cattle although the symptoms were more pronounced. A daily dose of 300 mg for five consecutive days (about 5 mg/kg/day), as recommended by van STEENIS (1951) against giardiasis in man, apparently is generally tolerated without symptoms of intoxication.

III CRITICAL SURVEY OF METHODS WHICH HAVE BEEN USED FOR TESTING THE EFFICACY OF FASCIOLICIDES AS MENTIONED IN THE LITERATURE

As has appeared from the foregoing chapter, the efficacy of drugs against *F. hepatica* has been determined by a few simple procedures.

The methods of screening drugs by determining whether they are able to exert lethal action on *F. hepatica in vitro* at very low concentration within a given lapse of time, or whether they affect the parasite as determined by the kymographic technique, would seem to be of value because great numbers of drugs can be quickly tested at comparatively low costs. These *in vitro* tests have, however, a great drawback: compounds which are active *in vitro* do not need to have any effect against the parasite *in vivo*. Several examples thereof can be found in the reviews given by LÄMMLER (1955, 1956), who also mentions hexachloroethane and male fern extract as examples of preparations which are active *in vivo* but comparatively inactive *in vitro*. One of the causes of the discrepancies between the *in vitro* and *in vivo* results might be the fact that liver flukes in the host are probably not poisoned by the presence of the drug in the medium wherein they live, *i.e.* the bile, but by the sucking of blood which contains the fasciolicide. VAN GREMBERGEN (1951) and JENNINGS *et al.* (1955, 1956) have presented evidence that *F. hepatica* sucks blood from the host, and LIENERT (1959*b*, 1959*a*, 1960, 1960*c*) has reported that *F. hepatica*, implanted under the skin of rats, was killed by oral administration of carbon tetrachloride, hexachloroethane, filmaron, or hexachlorophene to the host, obviously by mediation of the blood.

Small laboratory animals, especially rabbits and rats, experimentally infected with *F. hepatica*, have been used by several workers for selecting fasciolicides. The efficacy of drugs can be determined by killing the animals after treatment and by examining the liver and the gall-bladder for the presence of living flukes. The faeces of the experimental animals can also be examined for the presence of fluke eggs before and after treatment. LIENERT (1960*a*, 1960*b*) has developed a screening test which presents several advantages. In this method the effect of drugs on *F. hepatica*, implanted subcutaneously in rats, is determined.

The use of small laboratory animals has a disadvantage. The chemotherapeutic index of a drug is often very different from that which is found if the same drug is applied for the treatment of fascioliasis in other hosts. Carbon tetrachloride for instance was found by LÄMMLER (1956) to have a chemotherapeutic index of 25.0 on oral administration to rabbits, and hexachloroethane of >6.1. These indices are

obviously much lower when the drugs are administered to cattle. LÄMMLER (1960) found very different chemotherapeutic indices for 1,4-di(trichloromethyl)benzene when these were determined for rats, rabbits, sheep and cattle: the indices appeared to be 46.6, 4.9, >10, and >3 respectively. Apart from the price, it is the chemotherapeutic index which determines the usefulness of a drug for the treatment of fascioliasis in sheep and cattle.

LÄMMLER (1955, 1956) has developed a method to determine the relative activity of drugs by killing infected rabbits two days after treatment, and by measuring the extent of necrosis from the hind ends of the liver flukes in forward direction. One wonders whether the extent of the necrotic portions of the worms is also a function of time, and whether the rapidity at which a therapeutic level is reached in the blood, or possibly in the bile of the rabbits, influences the results.

For the determination of the efficacy of drugs against *F. hepatica* in farm animals generally two different methods have been used:

1. *Post-mortem* examination of the liver and gall-bladder for the presence of living flukes after treatment,
2. Examination of faecal samples collected before and after treatment for the presence or absence of eggs of *F. hepatica*, or estimation of the number of eggs in those samples.

Often a combination of both methods has been employed. OLSEN (1948) used for sheep the first method in combination with examination of the entire output of faeces after treatment as well as of the intestinal contents for the presence of liver flukes after slaughter, so that he was able to calculate the percentage of liver flukes which had been removed or killed by the drugs.

The reliability of the first method depends on the care with which the liver is examined, and one wonders how many flukes will be overlooked in the large livers of cattle and sheep. Probably often several flukes remain unnoticed, especially in enlarged and indurated livers. If the experimental animals are slaughtered more than a week after treatment, dead flukes can easily be distinguished from living ones, but slaughter within a few days after medication may present difficulties in this respect. The regeneration potential of partially affected flukes seems to be unknown, and complete immobility of the parasites when brought in physiological saline or bile at body temperature does not prove their death. Piperazine can cause paralysis in certain nematodes. Moreover, STANDEN (1953) has observed that the trematode *Schistosoma mansoni* in mice is rendered immobile after administration of lucanthone or antimonials to the host. Affected worms had lost muscle tone to a greater or lesser degree, and they moved but sluggishly, if at all, when placed in warm physiological saline. It further appeared that a number of schistosomes in mice recovered from the initial toxic effects unless very large doses of the drugs were given. The possibility of a similar action of drugs on *F. hepatica* can not be excluded, and the presence or

absence of muscular contractions should not be accepted as the only means to distinguish between life and death of the parasites.

The faecal examination has to be treated at some length, for application of this method has apparently often resulted in misleading conclusions on the efficacy of anthelmintics.

The interpretation of the results of faecal examination presents many difficulties. The following factors may be responsible for either over- or underestimation of the efficacy of a treatment against mature liver flukes.

a. Application of an unsuitable technique

The failure to find eggs of *F. hepatica* in the faeces needs not inevitably mean that no egg laying mature flukes are present. Often the number of eggs in the faeces, especially in those of cattle, are so small that a very sensitive concentration technique has to be employed to detect their presence.

b. The quantity of faeces examined

Light infections with mature flukes easily escape detection if an insufficient quantity of faeces is examined.

c. Suppression of egg production by anthelmintics

Most anthelmintics tend to affect ovulation of parasites at therapeutic, and sometimes considerably lower dosage. If faecal samples are examined within a few weeks after treatment, eggs of the parasites may be absent in the faeces, or present only in very low number, because of temporary suppression of egg production of surviving flukes. It follows that examination of the faeces too soon after treatment may result in overestimation of the efficacy of the drug. The possibility of permanent sterilisation of surviving flukes should also be considered.

It has been postulated that liver flukes, which have lived for several years, may become senile and stop egg production.

d. Variation of the egg count

It is a well known fact that the number of parasite eggs in the faeces shows considerable variation. Temporary obstruction of bile ducts will exaggerate the variation of the number of liver fluke eggs. Thus examination of faecal samples, which have been collected from a group of animals only once after medication, as has apparently often been practised, easily leads to wrong conclusions about the effect of treatment. For example, the percentage of animals which have stopped passing eggs in their faeces will be overestimated when only one faecal sample per animal is examined after treatment, since a number of animals found negative for eggs in their faeces will be found positive when faecal examination is repeated several times.

e. Sampling too late after treatment

If animals are treated which have been out of doors within the last two months, immature flukes are likely to be present. Since these young flukes generally escape the action of fasciolicides, they become mature and commence producing eggs after treatment. Thus examination of faecal samples collected from such animals several weeks after treatment tends to cause underestimation of the effect of a drug on mature flukes.

f. Retention of eggs in the host

The cholangitis caused by *F. hepatica* often interferes with the free flow of bile. Dead flukes and eggs can be trapped in the bile ducts for a considerable length of time. When dead flukes eventually disintegrate their contained eggs are liberated. Further the gall-bladder probably often retains eggs for a long period. It is therefore possible occasionally to find eggs in the faeces of animals which no longer harbour living mature flukes, even if a few weeks have elapsed after treatment. This is also a reason why one should not rely upon examination of only one or a few faecal samples after treatment.

g. Fluctuation of the number of eggs within a day

The present writer has discovered a fluctuation of the number of eggs of *F. hepatica* in cattle faeces within a day. This phenomenon will be dealt with later in this publication. It appears that comparison of egg counts of different faecal samples of cattle is permissible only if the samples are collected at the same time of the day. Only a few authors have taken this circumstance into account. Sampling before and after treatment at quite different times inevitably gives rise to erroneous conclusions about the efficacy of treatment so far as cattle are concerned.

Unfortunately many authors appear not to have appreciated the importance of one or more of these factors; hence their conclusions about the activity of drugs based on faecal examination should be regarded with reserve.

The presence or absence of eggs can be used only as a criterion for distinguishing incomplete from complete effectiveness of a treatment against mature flukes. Egg counts are of more value because they offer the possibility of obtaining an indication of the degree of efficacy of treatment.

Although it has become common practice to express the results of egg counts as numbers of eggs per gram of faeces, the results are often expressed in terms such as "many eggs", "small numbers of eggs" etc., which can not be compared with the results of other workers. It even seems doubtful sometimes whether any counting of eggs in a measured quantity of faeces has been carried out.

Several techniques have been described for the determination of the number of fluke eggs per gram of faeces. Since the number of eggs is often relatively small,

especially in cattle faeces, it is necessary to separate the eggs from the bulk of faecal material before they can be counted. This is effected by means of fractionated sedimentation, sedimentation in a current of water, application of solutions with a high specific gravity, or fractionated sieving. Most of these concentration techniques do not allow quantitative collection of the eggs, and generally an inconstant percentage of the eggs is lost. The attachment of eggs to the vessel walls, to light faecal particles, and to small air bubbles often interferes with proper sedimentation. Flotation fluids cause distortion of the eggs within a few minutes; sometimes the operculum is lost, and a number of eggs do not float. Moreover, distorted eggs are more difficult to identify, and they are more easily overlooked than normal eggs.

It is remarkable that these techniques are usually accepted in the belief that the resulting egg counts are correct. Reproducibility of the counts is very seldom demonstrated. Although the reliability of an egg counting method can be tested by determination of recovery rates after the addition of known numbers of eggs to faecal samples, unfortunately it is not usual to present such data. The very low egg counts which have been recorded by some authors suggest that the techniques they have used have not been satisfactory.

IV DEVELOPMENT OF A NEW METHOD FOR DETERMINING THE ACTIVITY OF DRUGS AGAINST *FASCIOLA HEPATICA* IN CATTLE AND SHEEP

1. Development of a new egg counting technique

The writer has not been able to obtain consistent results with some of the described methods for reasons which have already been mentioned. Therefore a method was tried, which had been developed by Willmott & Pester (1952) for counting the eggs of *Paramphistomidae* in cattle faeces. This technique is based on selective sieving. The eggs are collected from the faeces by washing a faecal suspension through a nest of sieves, and later through No. 16 bolting silk, which retains the eggs. This technique was found by the writer to be unsatisfactory for the quantitative collection of *Fasciola hepatica* eggs, since a number of these appeared to pass through No. 16 bolting silk. It was found, however, that *F. hepatica* eggs can be quantitatively collected on woven metal gauze with meshes no wider than 0.050 mm. Thus a method was developed which was based on fractionated sieving of a faecal suspension, and a satisfactory technique was designed for the estimation of the number of eggs in the obtained egg suspension. As this method has already been described in detail (Dorsman, 1956), it suffices to mention the principal features only.

A few hundred grams of cattle faeces are thoroughly mixed with an equal quantity of water in a 1 liter conical measuring beaker to a homogeneous suspension with a screw propeller coupled to a powerful laboratory stirrer. While the mixture is still stirring, amounts of about 1 ml are taken out of the beaker until 6 ml suspension are collected, which correspond to 3 grams of faeces. This sample is washed with water on a sieve with meshes of about 0.16 mm. The fluke eggs pass through the sieve, and they are collected quantitatively (together with other fine faecal particles) on a small sieve with meshes of no more than 0.050 mm. The debris on this fine gauze is stained with methylene blue, which does not change the yellow-brown colour of the *Fasciola* eggs. The material on the small sieve, which contains the eggs, is now transferred to a cylindrical vessel with 24 ml of water. Six ml of a solution of carboxymethylcellulose are added, and even distribution of the eggs is obtained by magnetic stirring. Part of the fluid is now discharged from the vessel through a narrow tube into a counting slide by means of air pressure. The stirring is continued during this operation. As the carboxymethylcellulose slows down the sedimentation rate of the eggs, they become evenly distributed within the counting chambers. The eggs are counted in the squares of the two compartments of the counting slide, each corresponding with a volume of

1 ml. The mean number of eggs within the squares, multiplied by ten, corresponds to the number of eggs per gram of faeces.

As the correctness of the resulting egg counts is not proved by their reproducibility alone, the method was checked by adding known numbers of eggs to faecal samples. The results have shown that the added eggs were quantitatively recovered, and thus it was proved that the method yields reliable results.

2. Variation of the egg count

Reduction in the number of *Fasciola* eggs in the faeces, as a result of treatment, can be ascertained only when the number of eggs in the faeces of an animal represents a reasonably constant value. In order to investigate the practicability of egg counts for detection of fasciolicidal activity, the variation of the egg count of faecal samples, collected from individual animals, was determined at short and long time intervals.

a. Cattle

The changes in the number of eggs per gram of faeces within a day were determined in housed and grazing cattle. Faecal samples were collected by manually induced defecation with intervals of one hour. Whenever possible only the last few hundred grams of faeces, which passed out of the rectum, were used. The number of eggs per gram in each of these samples was assessed by means of the described technique.

The results have been depicted in Fig. 1 and 2, in which the number of eggs per gram of faeces (E.P.G.) has been plotted against time. The different curves show a remarkable conformity: in all cases the number of eggs increased considerably in the morning, a maximum was reached at about 1.30 p.m. Central European Time, and thereafter the number of eggs decreased. The results of examination of faecal samples, which have been collected from a housed animal during a period of 24 hours, show that the count remained virtually constant at a relatively low level during the night (Fig. 1: curve A4).

No counts have been recorded in curve A1 at 12.30 p.m., in curve B1 at 8.30 a.m., and in curve B3 at 10.30 a.m. because at those times the rectum was empty.

Curves B1, B2 and B3 show only that a rise and fall of the number of eggs occurred. Further, hardly any value should be attached to the shape of these three curves, because the accuracy of the counts is low in cases of such low faecal egg-concentrations.

When the curves in Fig. 1 and 2 are compared, it will be noticed that the counts vary more widely near the middle of the day than early in the morning or late in the afternoon. At 8.30 a.m. for instance, the E.P.G. values in ten of the eleven curves range only between 5 and 30, while in seven out of these ten curves this value appears to be 5 E.P.G. when in curve B1 the count of 9.30 a.m. is substituted for the missed one of 8.30 a.m. The counts at 1.30 p.m. in these ten curves, however, vary between the limits of 5 and 75 E.P.G.

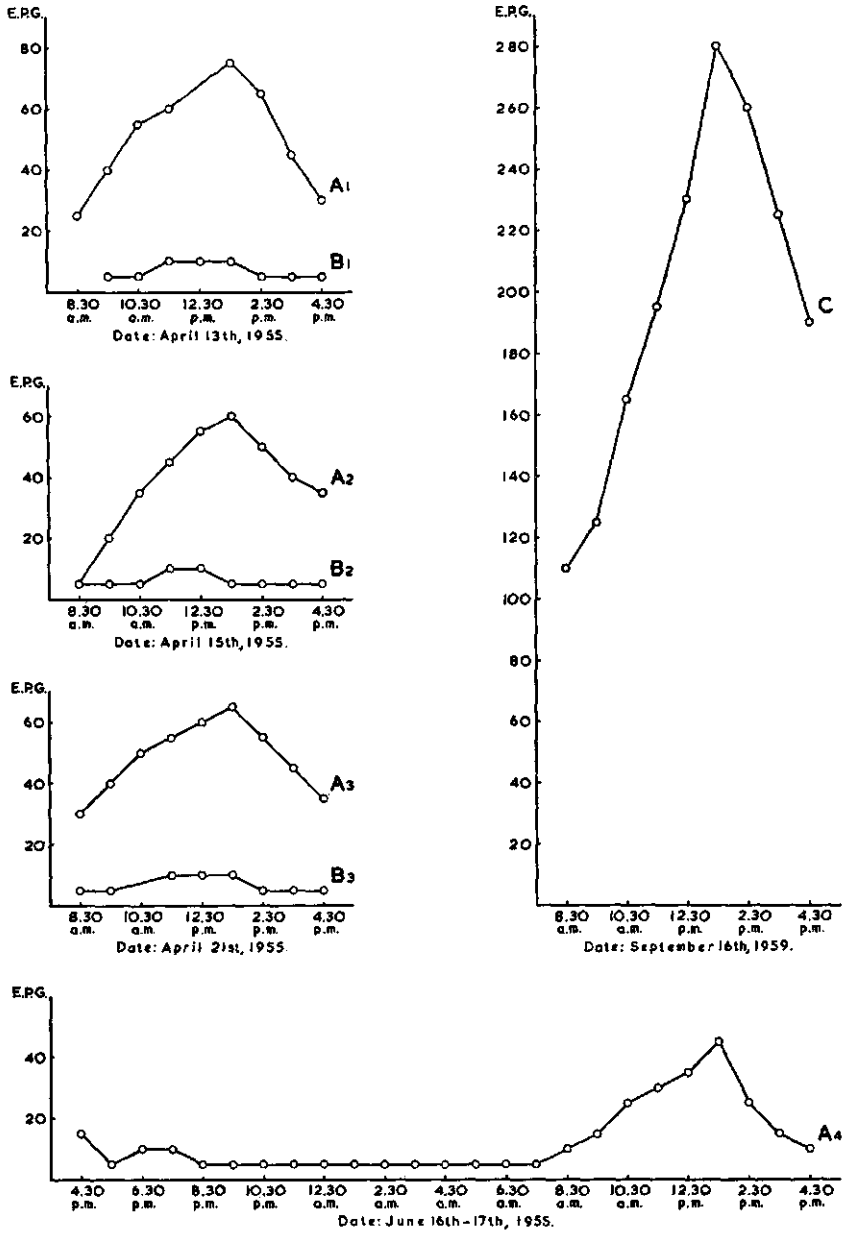


FIG. 1. Number of eggs of *Fasciola hepatica* per gram of faeces (E.P.G.), collected at intervals of one hour, of housed cattle.

A: Heifer No. 2273; B: Heifer No. 2272; C: Cow No. 369941

Nearly all these results have already been described (DORSMAN, 1956*a*), and another example of the fluctuation of the egg count within a day has been given later (DORSMAN, 1960).

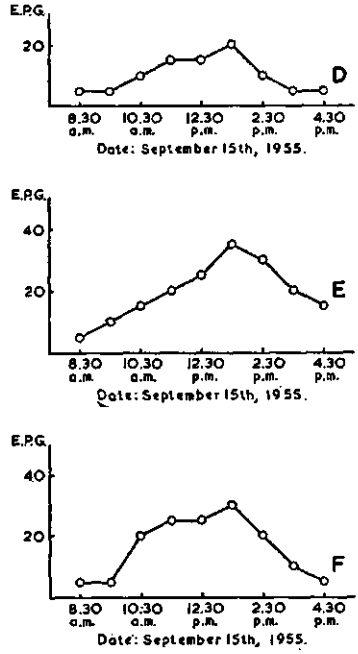


FIG. 2. Number of eggs of *Fasciola hepatica* per gram of faeces (E.P.G.), collected at intervals of one hour, of grazing cattle.
D: Heifer No. 1536; E: Heifer No. 1666; F: Heifer No. 9930

As the fluctuation of the number of *Fasciola* eggs in the faeces can be assumed to be repeated in a similar way every day, the phenomenon has some consequences of practical importance. In the first place, it is clear that comparison of pre- and post-treatment egg counts is only justified when faecal samples are collected from the rectum at the same time of the day on both occasions. Secondly, it appears that the quantitative differences in egg output in different animals can be more readily distinguished when the eggs are counted in fresh faecal samples collected in the beginning of the afternoon, than when samples are used which have been obtained from the rectum early in the morning or late in the afternoon. Thirdly, it can be concluded that the day-to-day variation in the number of eggs in the faeces of an animal, which is sampled once a day, will be smallest when the samples are always obtained from the rectum at the same time.

b. Sheep

Faecal samples were collected from the rectum of four sheep with intervals of one hour. This was carried out in one of the animals on eight different days. Variation in egg count between 8.30 a.m. and 4.30 p.m. appeared to be irregular. A fluctuation in

the number of eggs was not found. Collection of fresh faecal samples at a fixed hour seems therefore not to reduce the variation in daily egg count.

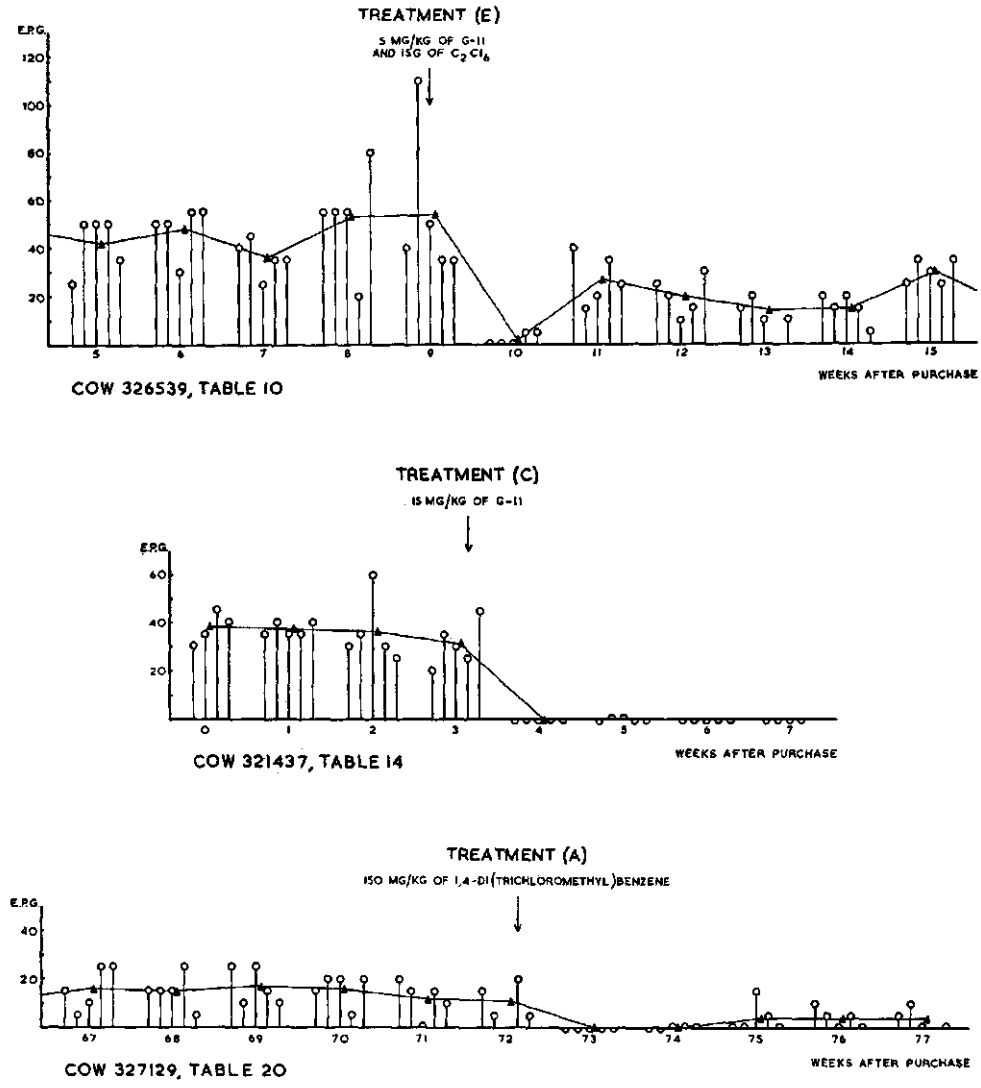


FIG. 3. Effect of treatments on the number of eggs of *Fasciola hepatica* per gram faeces (E.P.G.) in cattle

- ▲ weekly mean count
- ⌓ no eggs found within the squares of the counting slide, but eggs present in 3 grams of faeces
- ⌔ no eggs found in 3 grams of faeces

3. Methods used for determining the activity of fasciolicides

a. Faecal examination

The above technique for the assessment of the number of *Fasciola* eggs per gram of faeces has been used throughout these investigations. When no eggs were found within both squares of the counting slide, the entire amount of 30 ml, derived from 3 grams of faeces, was examined in shallow rectangular dishes under a dissecting microscope for the presence or absence of eggs. In a number of samples of sheep faeces, however, the search for eggs in 3 grams of faeces was not carried out when none were detected within the two squares of the counting slide. In these cases the bottom surface outside the square of each counting chamber was also examined. The volume of each chamber of the counting slide is 2 ml, whereas the volume from which eggs may sediment on the surface within a square is 1 ml.

In view of the practical consequences of the diurnal fluctuation of the number of *Fasciola* eggs in cattle faeces, the routine was adopted always of collecting faecal samples from cattle by manually induced defecation between 1.30 and 2.00 p.m., and to use only the last few hundred grams which passed out of the rectum. Although a daily fluctuation of the number of *Fasciola* eggs in sheep faeces was found not to occur, for practical reasons faecal samples were always collected from the rectum of experimental sheep at the same time as those of cattle.

When faecal samples were collected in this way from a given animal five times a week (Monday to Friday inclusive), the mean of the five counts generally appeared to represent a fairly constant value compared with the changes which occurred after treatments with fasciolicides (Fig. 3–5). When the number of eggs in the faeces was so low that, on one or more occasions, no eggs were found within a square of the counting slide, the mean weekly count was obtained in the following way. The total number of eggs which was found within the squares of the counting slide after examination of the daily faecal samples within a week, was divided by the number of examined squares, and this quotient was multiplied by ten. When for instance the following numbers of eggs were found within the two squares of the counting slide:

Monday	0 and 0
Tuesday	0 and 0
Wednesday	0 and 1
Thursday	1 and 0
Friday	0 and 0

the total number of eggs was 2. The mean number found within a square was 2/10, and the mean count was $2/10 \cdot 10 = 2$ eggs per gram. When the sample of Monday would not have been obtained, the mean weekly count would have been recorded as 3 ($2/8 \cdot 10 = 2.5$, rounded off: 3). In the presented graphs daily counts as those of Wednesday or Thursday of the example have been depicted as 5 eggs per gram, just

to indicate that one egg has been found in the counting slide, and not as to give an assessment of the number of eggs per gram in these samples.

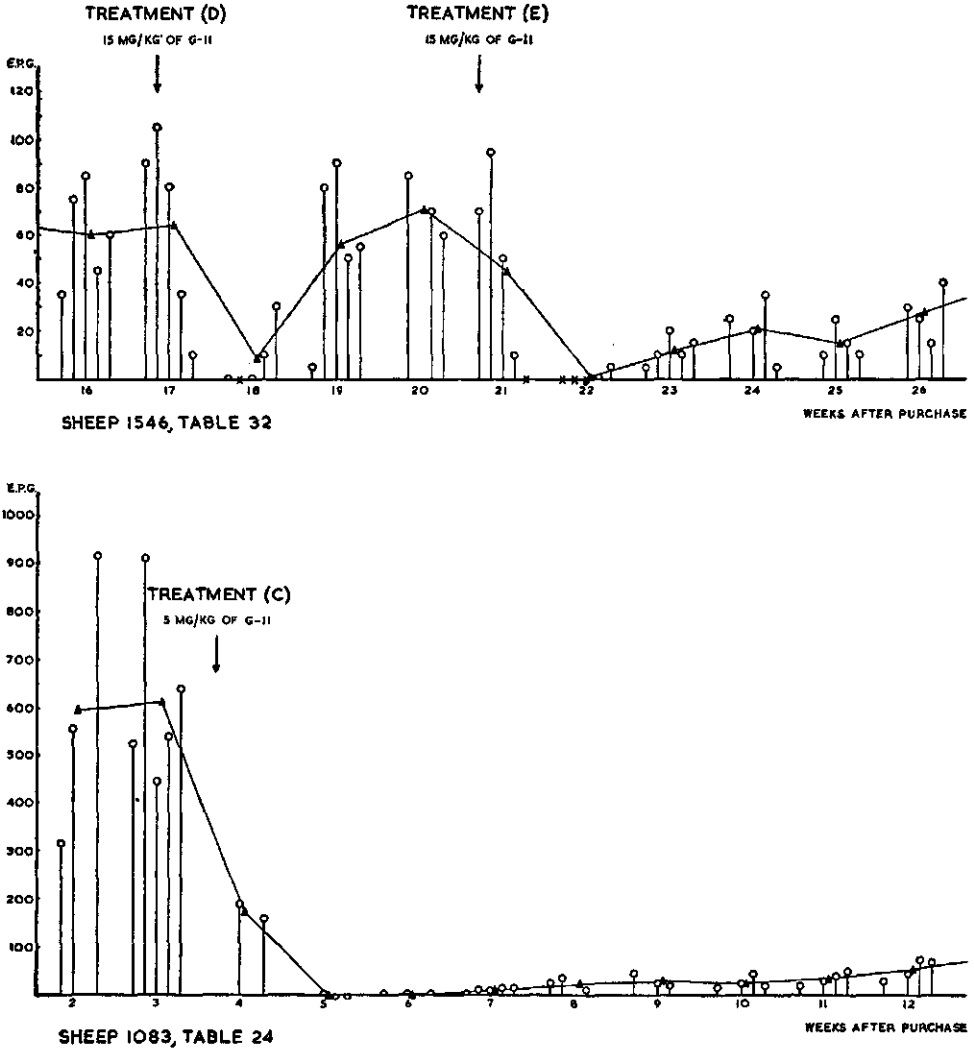


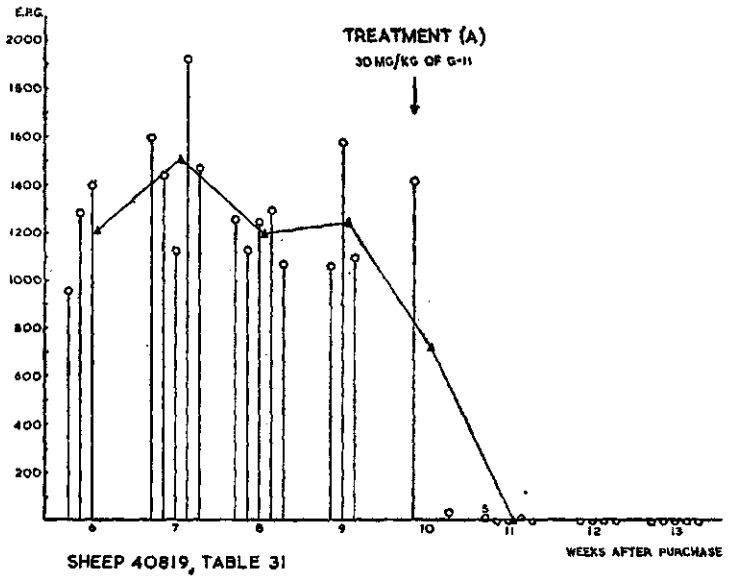
FIG. 4. Effect of treatments on the number of eggs of *Fasciola hepatica* per gram faeces (E.P.G.) in sheep

▲ weekly mean count

⌒ no eggs found within the squares of the counting slide, but eggs present in 3 grams of faeces

⌣ no eggs found in 3 grams of faeces

× no eggs found within both compartments of the counting slide



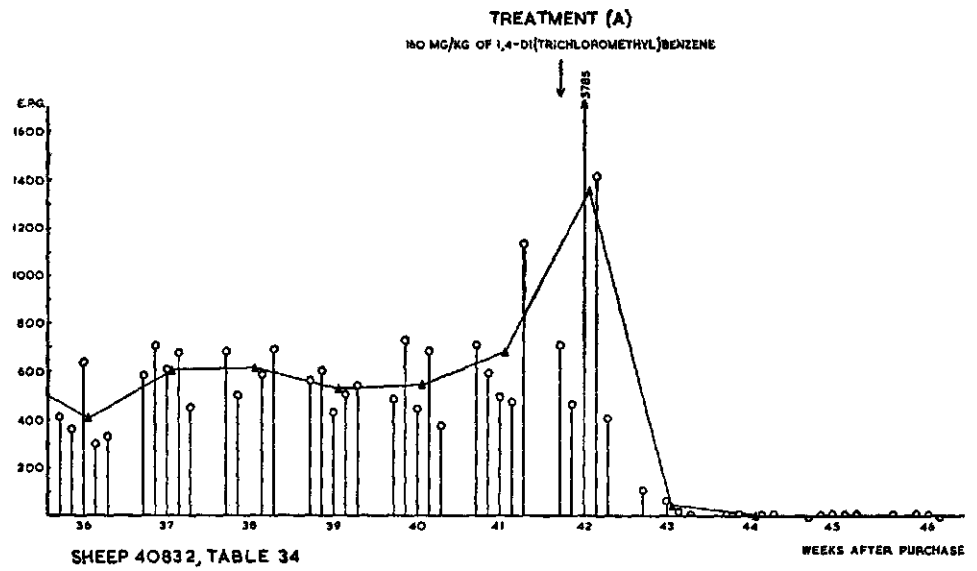


FIG. 5. Effect of treatments on the number of eggs of *Fasciola hepatica* per gram faeces (E.P.G.) in sheep

▲ weekly mean count

⌒ no eggs found within the squares of the counting slide, but eggs present in 3 grams of faeces

⌒ no eggs found in 3 grams of faeces

On several occasions a weekly mean count of 5 eggs per gram, or even lower, as obtained in the described way, was maintained for several consecutive weeks, without showing great variation. When a highly active fasciolicide was administered to animals, which showed such a very low and relatively constant weekly mean count, a fall in the mean weekly count was observed, which considerably exceeded the variation that occurred before medication. For these reasons it would seem that recording the very low mean counts is justified, because they appear to be of value as a reflection of the number of eggs produced by the worms. It should be realized, however, that the accuracy of the counts is very low when only a few eggs are present per gram of faeces.

By determining these weekly averages during periods covering several weeks before and after treatment, results were obtained, which are considered to give very valuable information on the activity of drugs against *F. hepatica* in cattle and sheep. This procedure, which has been practised throughout the course of these investigations, has several advantages:

1. The variation in egg count is greatly reduced.
2. A temporary decrease in the number of eggs can be easily distinguished from a lasting reduction, which is not possible when egg counts are carried out only occasionally.
3. A rise in egg count, due to surviving young flukes, which commence producing eggs after treatment, does not escape detection.
4. The method is sensitive: a slight effect of drugs on the flukes, consisting only of a temporary reduction in egg production, can be recognised. This is important for the screening of drugs, for after detection of that effect one can investigate chemically related compounds.
5. The degree of reduction in the mean egg count can be used as a rough measure for the anthelmintic activity of drugs.

Following an adaptation period of a few weeks after purchase, the mean weekly count often remained fairly constant from week to week, with a tendency to decrease gradually, in untreated housed animals, when the diet remained unchanged, and in the absence of great numbers of immature flukes, which eventually could grow mature. In some animals, however, considerable variation sometimes occurred, especially in those which were heavily infested. When administration of a drug was followed by a fall in weekly mean count, it had therefore to be established whether the fall was coincidental or due to the action of the drug. This was done by repeating the same treatment more than once.

b. Post-mortem examination

Whenever possible *post-mortem* examination of the experimental animals has been carried out. Within a few hours after slaughter the livers were cut into slices of about an inch thick, and the bile ducts, which had been opened in this way, were carefully examined for the presence of flukes. The gall-bladders were also examined. Nearly

always the animals were slaughtered several weeks after treatment, and living flukes could easily be distinguished from dead ones. The living flukes were of normal appearance, and showed muscular contractions when brought in physiological saline at body temperature, whereas the dead parasites were discoloured, had lost their tonus, and showed disintegration of internal structure.

c. Experimental animals

Several authors have found that the liver lesions resulting from chronic liver fluke infection tend to diminish the efficacy of medication. The original plan to use experimentally infected animals was therefore abandoned, and infected cows and sheep were bought. The cows, nearly all of the Friesian breed, were estimated to be usually between seven and ten years old. In view of the liver lesions which were severe enough to warrant condemnation of all livers by the meat inspectors at the abattoir, it seemed that nearly all cattle had been infected for years. The purchased sheep, of the Texel breed, were of different ages, but usually about one year old. Many of the sheep were suffering from severe chronic fascioliasis.

V PREPARATIONS TESTED FOR ACTIVITY AGAINST *FASCIOLA HEPATICA*

Cattle were employed for the screening of preparations in view of the economic importance of the disease in cattle in the Netherlands, and because the known treatments of these animals against fascioliasis appeared to be unsatisfactory. Sheep were used at a later stage of the investigation to test a few preparations.

Although hexachloroethane seems to be the most commonly employed drug against liver fluke in cattle, this compound has not been included in these tests, because faecal examinations had shown us that the efficacy of this drug is often very poor in cows suffering from chronic fascioliasis.

The preparations included in these investigations have been chosen for several reasons. Some were known to possess activity towards *F. hepatica*: *carbon tetrachloride*, "*Vitan*", *Freon 112*, *hexachlorophene* and "*Hetol*", while *kamala* had been found by ALICATA (1941, 1946) to be of value against *Fasciola gigantica* in cattle.

Phloroglucinol and *N-butyryl phloroglucinate* were tried because phloroglucinol, and methylated derivatives thereof, have been found among the products obtained by partial degradation of the anthelmintic principles of male fern, kamala, and kousso, while butyric acid has also been obtained from male fern and kousso (SCHMIDT, 1923).

Some of the preparations were chosen because their activity against other plathelminths had been reported or suggested:

2-acetylamino-5-nitrothiazole, against *Schistosoma mansoni* (CUCKLER *et al.*, 1955).

stannous oxide and *stannous chloride*, against *Schistosoma mansoni* (MAUZÉ & ARNAUD, 1954; DESCHIENS et al., 1956, and others). Tin oxide and tin chloride have also been found to be effective against intestinal tapeworms (LEPINAY, 1933, and others).

di-n-butyl tin dilaurate, against tapeworms in chickens (KERR, 1952, and others).

di-n-octyl tin dilaurate and *di-n-octyl tin dichloride*, against the tapeworm *Hymenolepis fraterna* in mice and rats (HARANT *et al.*, 1957).

chloroquine, against *Opisthorchis viverrini*, a liver fluke occurring in man (SADUN *et al.*, 1955).

dichlorophene (G-4), against *Thysanosoma actinioides*, a tapeworm occurring in the bile ducts, pancreatic ducts, and small intestine of ruminants (RYFF *et al.*, 1949, and others), and against intestinal tapeworms (CRAIGE & KLECKNER, 1946, and others).

hexachlorophene (G-11), against tapeworms in chickens (KERR, 1948, and others), against the cercariae of *Schistosoma mansoni* (HUNTER *et al.*, 1956), and against *Fasciola hepatica* in sheep (HIRSCHLER, 1957).

hygromycin B, against *Thysanosoma actinioides* (KELLEY *et al.*, 1960).

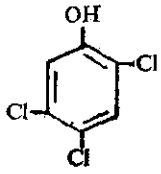
menthol, against helminths *in vitro* (ABDEL-MALEK, 1951). According to FRERICKS *et al.* (1938) menthol acts as a cholagogue if administered orally, and oil of peppermint (*l*-menthol being the main constituent) has been employed for treating patients with gall-stones.

T-pol 410 (Shell), against *Carmyerius dollfusi*, a rumen fluke of zebus in Madagascar (DAUMAS & GRETILLAT, 1958). T-pol 410 is a mixture of secondary sodium alkylsulphates.

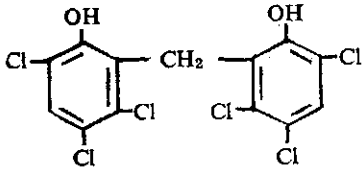
A number of miscellaneous preparations has also been tested.

Apart from dichlorophene (G-4), three bisphenols were tried which are closely related to hexachlorophene (G-11): *2,2'-thiobis (3, 4, 6-trichlorophenol)* or compound G-11S, *2,2'-methylenebis (4, 6-dichlorophenol)* or compound G-5, and *2,2'-thiobis (4,6-dichlorophenol)* or bithionol. The latter compound, like hexachlorophene, has been found active against schistosome cercariae (HUNTER *et al.*, 1956). The chlorinated phenol, *2, 4, 5-trichlorophenol* is used for the synthesis of hexachlorophene (G-11).

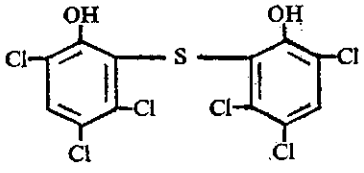
These phenols have the following structure:



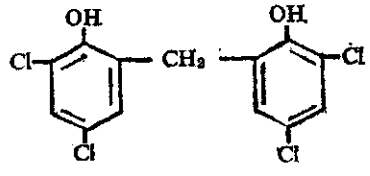
2,4,5-trichlorophenol



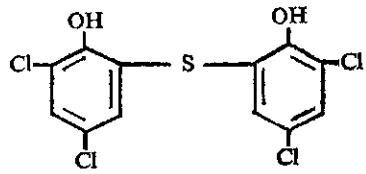
2,2'-methylenebis (3,4,6-trichlorophenol)
Hexachlorophene (G-11)



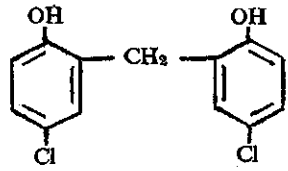
2,2'-thiobis (3,4,6-trichlorophenol)
G-11S



2,2'-methylenebis (4,6-dichlorophenol)
G-5



2,2′-thiobis (4,6-dichlorophenol)
Bithionol



2,2′-methylenebis (4-chlorophenol)
Dichlorophene (G-4)

Additional preparations studied included "*Felamine*" which contains *cholic acid* and *hexamethylene tetramine*, and is used as a cholagogue and bile antiseptic (PINKHOF *et al.*, 1951); *sunflower-seed oil*, because it induces an increase in the rate of cholic acid secretion with the bile (LEWIS & VOLKS, 1958); and *lidocaine*, a local analgesic forming one of the constituents of the preparation employed against *Fasciola hepatica* in cattle by KOVÁCS (1959).

The following compounds were also tried: *resorcinol*, *sodium lauryl sulphate*, *procaine hydrochloride*, *sodium pentachlorophenate*, *dinitro-o-cresol* and its ammonium salt, *1,8-dihydroxy-anthraquinone*, and *chlorhexidine hydrochloride*.

VI PREPARATIONS FOUND INEFFECTIVE AGAINST *FASCIOLA HEPATICA* IN CATTLE AT THE EMPLOYED DOSAGES

The following treatments did not result in an obvious decrease of the mean number of *Fasciola* eggs per gram of faeces.

Resorcinol
Calf 7642
Body weight: about 150 kg.
Dates of treatment: 18–20 II, 1958.
Dose: 18 II: about 20 mg/kg; 19 II: about 27 mg/kg; 20 II: about 20 mg/kg.
Administration: dissolved in water, given orally.

Cow 8902: vide Table 3, treatment (K).

2-Acetylamino-5-nitrothiazole
Heifer 8902
Body weight: about 380 kg.
Dates of treatment: 3–8 VI, 1957.
Dose: about 20 mg/kg on each of six consecutive days.
Administration: 50 g premix, which contained 15% Enheptin A, dispersed in water, administered by stomach tube, per day.

Heifer 8902: vide Table 3, treatment (A).

Stannic oxide (SnO_2)
Cow 2273
Body weight: about 550 kg.
Dates of treatment: 3–7 IX, 1956.
Dose: about 36 mg/kg on each of five consecutive days.
Administration: 20 g per day, suspended in water and administered by stomach tube.

Chloroquine sulphate
Cow 2273
Body weight: 560 kg.
Dates of treatment: 5–23 XI, 1956.
Dose: 5 XI and 6 XI: 10 mg/kg/day; 7 XI–23 XI: 5 mg/kg on each of 17 consecutive days.
Administration: "Nivaquine" tablets, crushed with mortar and pestle, dispersed in water, given by stomach tube.

Heifer 8902
Body weight: about 350 kg.
Dates of treatment: 1–6 IV, 1957.
Dose: 1 IV and 2 IV: about 10 mg/kg/day; 3 IV–6 IV: about 5 mg/kg on each of four consecutive days.
Administration: as above.

Hygromycin B
Cow 420219
Body weight: about 650 kg.
Dates of treatment: 4–6 and 8–18 VIII, 1960 (altogether 14 days).
Dose: about 0.5 mg/kg/day.
Administration: the animal was drenched each day with a suspension of 60 g "Hygromix" (which contained 2.4 g hygromycin B per lb) in water.

dl-Menthol
Heifer 8902
Body weight: about 360 kg.
Date of treatment: 24 IV, 1957.
Dose: about 6 mg/kg.
Administration: 2 g, dissolved in alcohol, given by mouth.

Heifer 8902
Body weight: about 360 kg.
Date of treatment: 7 V, 1957.
Dose: about 30 mg/kg.
Administration: 10 g, dissolved in a mixture of alcohol and glycerol, given by mouth.

Sodium lauryl sulphate
Cow 326686
Body weight: about 580 kg.
Dates of treatment: 7 and 9 XII, 1959.
Dose: 7 XII: about 17 mg/kg; 9 XII: about 51 mg/kg.
Administration: 7 XII: 10 g dissolved in water, and about 30 ml "Tymasil" (Brocades). an anti-bloat remedy on the base of silicones; 9 XII: 30 g dissolved in water, and about 5 ml "Tymasil". Both doses were administered orally.

T-pol 410 (Shell)
Cow 326686
Body weight: 585 kg.
Date of treatment: 30 XII, 1959.
Dose: 35 g of a 21 % solution.
Administration: the solution was given orally, together with a few drops of "Tymasil".

Cow 326539
Body weight: about 600 kg.
Date of treatment: 9 II, 1960.
Dose: 70 g of a 21% solution.
Administration: orally.

Cow 326539
Body weight: 604 kg.
Date of treatment: 7 III, 1960.
Dose: 170 g of a 21% solution.
Administration: the solution was given orally, together with 5 ml "Tymasil".

Cow 326686
Body weight: about 630 kg.
Date of treatment: 25 IV, 1960.
Dose: 300 g of a 21% solution.
Administration: the solution was given orally, together with 3 g "Tymasil".

2,2'-Methylenebis (4-chlorophenol), dichlorophene, G-4
Cow 5104
Body weight: 480 kg.
Date of treatment: 18 VII, 1958.
Dose: 63 mg/kg.
Administration: 60 "Dicestal" tablets, each containing 0.5 g dichlorophene, crushed and suspended in water, administered by stomach tube.

Cow 326686
Body weight: 669 kg.
Date of treatment: 30 VI, 1960.
Dose: 150 mg/kg.
Administration: a suspension of 100 g G-4 in water, prepared with sodium lauryl sulphate as a wetting agent, was administered by stomach tube. About 75% of the administered quantity of dichlorophene was purchased from LIGHT & Co.; the remaining portion was prepared from "Dicestal" tablets.

Cow 326686
Body weight: about 680 kg.
Date of treatment: 17 VIII, 1960.
Dose: about 44 mg/kg.
Administration: G-4, prepared from "Dicestal" tablets (melting point 159–160°C), solubilized in water with Tween 80, was administered by stomach tube. The

administered quantity of 1500 ml clear fluid contained 30 g dichlorophene, 300 g Tween 80, and 7.5 g orthooxyquinoline sulphate ("Superol").

2,2'-Methylenebis(4,6-dichlorophenol), G-5
Cow 458283: vide Table 2, treatment (F).

Cow 306493
Body weight: 642 kg.
Date of treatment: 28 X, 1958.
Dose: 30 mg/kg.
Administration: as above.

"*Felamine*"
Cow 5104
Body weight: about 480 kg.
Dates of treatment: 12–14 VIII, 1958.
Dose: about 15 mg hexamethylene tetramine/kg and 5 mg cholic acid/kg on each of three consecutive days.
Administration: 30 tablets "Felamine", crushed with mortar and pestle and suspended in water, given orally, per day.

Sunflower-seed oil
Cow 326539
Body weight: about 580 kg.
Dates of treatment: 7–9 XII, 1959.
Dose: 7 XII: 400 ml; 8 XII: 400 ml; 9 XII: 300 ml.
Administration: orally.

Lidocaine hydrochloride
Cow 217561
Body weight: about 650 kg.
Date of treatment: 18 II, 1960.
Dose: about 0.5 mg/kg.
Administration: 300 mg, dissolved in about 12 ml distilled water, injected subcutaneously.

Cow 420219
Body weight: about 600 kg.
Date of treatment: 2 III, 1960.
Dose: about 0.5 mg/kg.
Administration: 300 mg, dissolved in about 20 ml distilled water, injected subcutaneously.

Cow 217561
Body weight: about 650 kg.
Date of treatment: 14 III, 1960.
Dose: about 2.3 mg/kg.
Administration: 1500 mg, dissolved in about 20 ml distilled water, injected subcutaneously.

Procaine hydrochloride (Novocaine)
Cow 326686
Body weight: about 620 kg.
Date of treatment: 9 III, 1960.
Dose: about 1.6 mg/kg.
Administration: 1000 mg, dissolved in distilled water, injected subcutaneously.

Dinitro-o-cresol
Calf 7642
Body weight: about 160 kg.
Date of treatment: 28 IV, 1958.
Dose: about 10 mg/kg.
Administration: 1.6 g, suspended in water, administered by stomach tube.

Calf 7642: vide Table 2, treatment (A).

Cow 420219
Body weight: about 650 kg.
Date of treatment: 25 V, 1960.
Dose: about 8 mg/kg of the ammonium salt.
Administration: 5 g of the ammonium salt, partially dissolved and partially suspended in water, was administered by stomach tube.

1,8-Dihydroxy-anthraquinone
Calf 7642: vide Table 2, treatment (C).
The diarrhoea following medication accounts for the low mean egg count of the week of treatment. The rise in the number of eggs in the next week was probably due to the small food intake.

Chlorhexidine hydrochloride
Cow 9511
Body weight: 500 kg.
Date of treatment: 18 VII, 1958.
Dose: 40 mg/kg.
Administration: 20 "Hibitane" pessaries (I.C.I.), each containing 1 g chlorhexidine

hydrochloride, were dispersed in water with mortar and pestle, and administered by stomach tube.

Administration of a few preparations resulted in a decrease of the egg count, which was not clearly a result of medication. The fasciolicidal activity of the following preparations can therefore be considered as doubtful.

Kamala and *Phloroglucinol*

Cow 8902 received kamala from the 6th to the 9th January 1958, and phloroglucinol from the 14th to the 16th of the same month: Table 3, treatments (F) and (G). The latter treatment was followed by a marked drop in the number of eggs in the faeces, as is shown in Table 3.

The following treatments with these preparations, however, did not result in an effect on the egg count, which markedly exceeded the variations in untreated animals.

Kamala

Cow 8902: vide Table 3, treatment (D).

Cow 8902: vide Table 3, treatment (E).

Phloroglucinol

Cow 8902: vide Table 3, treatment (H).

Cow 8902: vide Table 3, treatment (J).

Calf 4139
Body weight: 182 kg.
Dates of treatment: 27–29 I, 1958.
Dose: 27 I: 20 mg/kg; 28 I: 35 mg/kg; 29 I: 50 mg/kg.
Administration: orally, dissolved in water.

Heifer 5112
Body weight: 486 kg.
Dates of treatment: 4–6 II, 1958.
Dose: 4 II: 20 mg/kg; 5 II: 35 mg/kg; 6 II: 50 mg/kg.
Administration: orally, dissolved in water.

The effect observed after treatment (G) of Table 3 could not be reproduced with higher doses of phloroglucinol. It seems therefore unlikely that the reduction in the egg count was caused by phloroglucinol. Since the number of eggs began to decrease

more than a week after the last administration of kamala, and as the other treatments with this drug did not affect the egg count, it seems also unlikely that kamala was responsible for the effect. For these reasons it may be concluded that kamala and phloroglucinol were inactive at the employed dosages, although the possibility can not be ruled out that phloroglucinol, given within a short interval after high doses of kamala, has affected the flukes.

n-Butyryl phloroglucinate

A temporary reduction in the number of eggs per gram of faeces has been observed three weeks after administration of the compound to calf 7642 (Table 2, treatment (B)). Since this reduction occurred after such a long interval, the writer is inclined to believe that the phenomenon has not been due to the action of the preparation.

Sodium pentachlorophenate

The treatment of cow 209812, mentioned in Table 4, seems to have resulted in a considerable reduction in the egg count. The animal was medicated on Monday, and thus an effect of the compound could have been expected to come to light in the week of treatment. The drop in number of eggs was, however, not reproduced when three other animals were treated with comparable doses of the same compound. The treatments with sodium pentachlorophenate, which did not result in an obvious decrease of the egg count, are the following.

Cow 361083: vide Table 2, treatment (G).

Cow 360413: vide Table 4, treatment (A).

Cow 326686
Body weight: about 650 kg.
Date of treatment: 25 V, 1960.
Dose: about 46 mg/kg.
Administration: by stomach tube, dissolved in water.

Stannous chloride ($SnCl_2 \cdot 2H_2O$)

Intramuscular injections of this compound seem to have affected egg production of the liver flukes in cow 8902: Table 3, treatments (B) and (C). The decrease of the number of eggs was gradual, and the number of eggs showed already a tendency to decrease the last two weeks before medication.

The treatment has not been repeated with other animals because the results were not considered as promising. It is therefore not clear whether stannous chloride possesses activity towards *Fasciola hepatica.*

2,2'-Thiobis(4,6-dichlorophenol), bithionol

The mean faecal egg count of cow 360406 showed a reduction following adminis-

tration of about 39 mg/kg of bithionol (Table 4, treatment (D)). Medication has been carried out on Friday, and hence the mean egg count of the week of medication cannot yet have been influenced by the preparation. It should be realized that the mean egg count showed already a gradual decrease in the course of the three weeks before treatment. The course of the egg count following treatment seems to be a continuation of the gradual decrease, and it seems very doubtful whether bithionol has influenced the course.

2,4,5-Trichlorophenol

A reduction in the number of eggs passed in the faeces has occurred the week following treatment of cow 308423 (Table 2, treatment (D)) with 10 mg/kg of 2,4,5-trichlorophenol. A dose of 20 mg/kg, administered to cow 458283 (Table 2, treatment (E)), however, has not been followed by a lowered egg count.

Signs of intolerance towards the employed preparations have been observed only on a few occasions.

Diarrhoea was observed the day following treatment of cow 326686 with 150 mg/kg of *dichlorophene*.

Calf 7642 suffered from profuse diarrhoea during the first six days following treatment with *1,8-dihydroxy-anthraquinone*. After this period the animal began to show symptoms of serious disease: the abdomen was swollen, and the appetite was very poor. The calf died twelve days after medication, and it appeared to have been suffering from pleuritis and a local peritonitis. The swelling of the abdomen had been caused by the presence of a huge amount of fluid.

The administration of *kamala* was regularly followed by a slight diarrhoea, which persisted for no more than a few days.

Cow 361083, which received *sodium pentachlorophenate* on three consecutive days, showed diminished appetite and reduced milk production during two days following the day of the last dosing. During this period the animal was less active than normally.

Toxic reactions have not been observed after any of the other treatments.

VII PREPARATIONS FOUND MORE OR LESS EFFECTIVE AGAINST *FASCIOLA HEPATICA*

Treatments with a number of preparations were followed by a reduction in the number of eggs in the faeces. This was apparently the result of injury inflicted upon mature flukes by the administered drugs.

Five of the preparations, belonging to this group, are known as fasciolicides: carbon tetrachloride, "Vitan", Freon 112, "Hetol" and hexachlorophene. Published data on the latter preparation only suggested its activity against *Fasciola hepatica* in sheep, whereas its effect on liver flukes in cattle was unknown when the experiments were commenced.

Other preparations, which have shown activity, and which appear to be unknown as fasciolicides, are:
hexachlorophene-piperazine complex,
2,2'-thiobis (3,4,6-trichlorophenol), and
alkyl tin compounds.

The treatments with the active preparations are mentioned hereunder. The evaluation of the results, and the toxicologic aspects, have been dealt with separately.

Alkyl tin compounds

Tests have been carried out with a calf, two cows, and a sheep. The treatments have been listed in Table I, and the relevant data have been given in the Tables 4, 5, 22a and 22b.

Carbon tetrachloride

Tests have been carried out with three cows and one sheep. Altogether eight doses have been given by the oral route, and five by intramuscular injection. The treatments have been listed in Table II, and the relevant data have been given in the Tables 6, 7, 8, and 23.

"*Vitan*" (Société Prolana, Lyon)

This proprietary preparation is a red stained fluid, which is delivered in hard capsules. The preparation has been tested on its efficacy in five cattle, and altogether nine treatments have been carried out (Table 9).

Vitan has been applied in doses as advised by the manufacturer. As it was not always easy to deposit the capsules at the back of the tongue, most treatments have been carried out by administering the fluid, which had been removed from the capsules. According to the directions for use the following precautions should be observed.

All food should be withheld during at least 15 hours (24 hours if possible) before treatment; after treatment starvation has to be continued during at least six hours. Thereafter only half the ration of hay should be fed during at least two days, and finally normal feeding can gradually be resumed. Concentrates and green crops should not be fed during some days before and after treatment.

These precautions, probably designed to minimize the risk of intoxication, have completely been observed in the case of treatments (D) and (E) of Table 9. In the other cases the precautionary measures have been limited. The animals which recieved the treatments (B), (C), (F), and (G) received no food on the day of medication, apart from a small quantity of hay about six hours after treatment. The second half of the day before, and the whole day after that of treatment, these animals have received only hay and water. In the case of treatments (A), (H), and (J), the animals in question have received only hay and water during the last six days before treatment. Food was withheld on the morning when the animals were treated, and a small quantity of hay was fed six hours after medication. Thereafter the daily hay ration of these three animals was gradually increased to the normal quantity.

Symmetric difluorotetrachloroethane, Freon 112

Freon 112 has been administered to two cows: Table 18.

2,2'-Methylenebis(3,4,6-trichlorophenol), Hexachlorophene, G-11

Tests have been performed with 34 cattle and 46 sheep. Altogether 40 doses have been administered to cattle, and seven of these have been given in combination with other compounds. These treatments have been listed in Table III, and the relevant data have been given in Tables 10–16.

The 46 sheep received altogether 65 treatments, which have been listed in Table IV. The data concerning the treatments of sheep with hexachlorophene have been given in Tables 24–33.

Medication of cattle and sheep has always been carried out by administering one single dose.

The cattle received a diet of hay and concentrates, and the sheep of alfalfa hay, often supplemented with oats. The daily ration of the experimental animals has never been altered either before or after treatment.

Hexachlorophene-piperazine complex

This drug has been administered to four sheep, and one of these animals received two doses. The treatments have been listed in Table V, and the concerning data have been given in the Tables 32 and 33.

2,2'-Thiobis (3,4,6-trichlorophenol), G-11S

Treatments have been carried out in three heifers: Tables 17a and 17b.

"*Hetol*" (Hoechst)

The product is a white powder, which contains 1,4-di(trichloromethyl)benzene in a concentration of 85%.

Tests have been performed with 12 cows, which altogether received 15 treatments, and with 6 sheep. The treatments of cattle have been listed in Table VI, and the concerning data have been given in the Tables 19, 20, and 21. The data concerning the treatments of the sheep have been given in Table 34.

According to the manufacturer, treatment has to be carried out when the animals have not yet been fed that day, and feeding can be resumed two to four hours after medication. The cows 17397 and 364024 were treated a few hours after the morning feeding, but in all other cases the mentioned directions have been strictly observed, and food was also withheld the second half of the day which preceded that of medication. Normal feeding of the sheep was maintained throughout the experiments.

VIII EVALUATION OF THE RESULTS OF TREATMENTS WITH ACTIVE DRUGS

1. General considerations

In this work the effectiveness of treatments has been evaluated by means of *post-mortem* findings, faecal egg counts, or a combination of both.

If animals are slaughtered within two or three weeks after medication, the interpretation of the results does not present great difficulties: the presence of flukes found on autopsy and the pre-treatment egg counts are the only factors to be taken into account. This also applies to the cases where *post-mortem* examination has been delayed to several weeks after treatment, while the egg count has remained unchanged during the last weeks. The counts following treatment then provide additional evidence on its effectiveness.

If animals are not slaughtered after treatment, egg counts represent the only indication of effectiveness, and their changes have to be interpreted carefully. The course of the egg counts is also of great importance in cases where animals have been slaughtered after an increase of the number of eggs in the faeces following a period in which the egg counts suggested that the host had effectively been cleared of mature flukes.

The figures of Table 1 show the relationship between the number of eggs per gram of faeces and the number of mature flukes found in the bile ducts of untreated cattle. Additional information on this subject can be gathered from the Tables 2 and 4, where results are recorded of cattle which have been slaughtered after the administration of drugs that probably did not affect the flukes to a great extent. It appears that generally a close correlation can be observed between the faecal egg counts and the number of mature flukes. This correlation would probably have come out even more clearly if faecal examinations could have been carried out over a longer period of time, and not only on three different days within one week.

In all cases the number of egg-laying *F. hepatica* appears to be considerably greater than the number of eggs of this parasite per gram of faeces. Such figures unfortunately cannot be presented from untreated sheep, because of the small number of available experimental animals. Lechner (1955) reported that the number of liver fluke eggs in the faeces of sheep corresponded with the extent of infestation.

When the faecal egg counts of sheep are considered, it must be assumed that the number of mature flukes as compared with the number of eggs per gram of faeces is much smaller than is the case in cattle. The comparatively small faecal output in sheep, and the low water contents of the faeces in these animals, are probably responsible for the relatively high egg counts often observed on examining sheep faeces.

Counts of a few thousands eggs per gram of faeces appeared invariably to be associated with severe clinical fascioliasis, while sheep with counts of a few hundreds eggs per gram were healthy.

An analysis of the course of the weekly average count of the experimental animals after medication shows, that seven different types can be distinguished on which the egg count is influenced by treatment.

I. The weekly average count remains practically unchanged.
Examples: calf 7642 after treatment (A), Table 2; sheep 1546 after treatment (F), Table 32.

II. A fall after treatment is followed by a constant level which is lower than that before treatment.
Examples: calf 1115 after treatment (A), Table 5; sheep 45693 after treatment (B), Table 27.

III. The weekly average count shows an abrupt fall after treatment, immediately followed by a rise, so that the original value is more or less approached within two to three weeks after treatment. Thereafter the count remains unchanged.
Examples: cow 326539 after treatment (E), Table 10; sheep 1117 after treatment (A), Table 25.

IV. An abrupt fall after treatment to a low value is followed within one or two weeks by a gradual rise, which is continued for at least several weeks.
Example: sheep 1133 after treatment (C), Table 25.

V. An abrupt fall after treatment to a very low value is followed by a period of a few weeks in which no more than very small numbers of eggs are found. Some weeks after treatment, however, the number of eggs begins to rise gradually.
Example: sheep 1083 after treatment (C), Table 24.

VI. Treatment is followed by an abrupt fall, and no eggs, or only occasionally very low numbers of eggs are found afterwards.
Examples: cow 208084, Table 13; sheep 1117 after treatment (F), Table 25.

VII. An abrupt fall after treatment is followed by a period of several weeks with low egg counts. Finally the eggs disappear.
Example: sheep 45687 after treatment (A), Table 33.

These types are depicted schematically in Fig. 6.

If the egg count followed type VI or VII, the treatment was considered to have been completely successful, and the animal was killed for autopsy. Examination of the bile ducts showed that this interpretation of the egg counts was correct: mature flukes were found in the bile ducts of only three out of 26 sheep in which the course of the egg count had conformed to type VI or VII, and the maximum number recovered

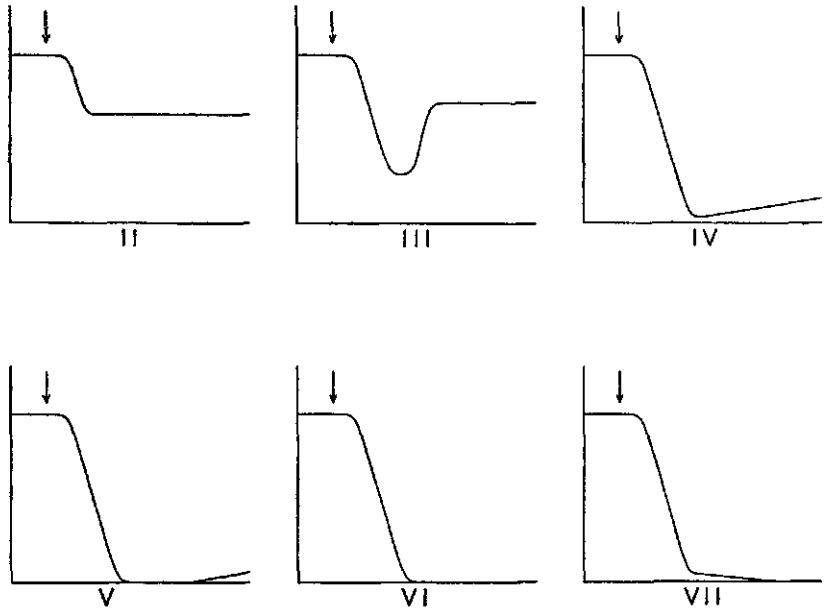


FIG. 6. Diagrams showing different courses of the weekly mean egg count following treatment

was two. In several cases animals were slaughtered within only a few weeks after medication, so that it was not possible to see whether the egg count followed type V or VI. In these cases also practically all mature flukes appeared to have been killed. The writer therefore believes that a reduction in the number of eggs in the faeces to a very low value, which is maintained for at least two weeks, indicates that all or nearly all mature flukes have been killed. A gradual rise of the count, as in type V can be interpreted as being the result of the survival of young flukes, which were immature when the host was medicated, and which started to produce eggs a few weeks later.

Type I doubtless indicates that treatment has had no effect, while type II strongly suggests that a number of mature flukes has been killed and that the egg production of the surviving mature flukes is not suppressed.

A course of the egg count as in type III seems to indicate that mature flukes have been temporarily damaged, and that few or no flukes have been killed.

Type IV can be regarded to be intermediary between the types III and V: a high percentage of the mature flukes has been killed; a small number of damaged flukes recover and start to produce eggs again, while in the meantime worms, which were nearly mature at the moment of treatment, also start to produce eggs. Later also younger flukes mature, and these further contribute to the rise of the egg count.

Type VII has been encountered on only a few occasions. There seem to be two explanations possible for this course of the egg count:

1. delayed action of the drug,
2. a very great number of mature flukes and eggs was present at the moment of treatment, so that it required several weeks before disintegrating flukes and ova were cleared from the bile ducts and gall-bladder.

The latter possibility is probably the explanation for the result obtained with sheep 1597, after treatment (A), Table 24. This animal however was slaughtered before the egg count could fall to a negligible level. Apart from sheep 1597, a similar result has only been observed after subcutaneous injection of hexachlorophene (sheep 45690 after treatment (C), Table 27) or of the hexachlorophene-piperazine complex (sheep 45687 and 45688, Table 33, where aluminum monostearate had been added with the purpose of trying to delay absorption of the drug), and never after oral administration. This fact suggests that in the cases of the last three sheep absorption of the drugs was indeed delayed.

If the explanations of the occurrence of the seven types on which the egg count is influenced by treatment are assumed to be true, it is understandable that the types I, II, and III may be modified by the presence of considerable numbers of nearly mature and immature flukes which have not been killed by medication. A new constant value of the egg count following treatment is then not maintained, but a gradual rise follows after a shorter or longer interval.

A few observations seem to support the view that types IV and V are the result of the presence of immature flukes, which escaped the action of the drug, and which developed to the mature stage after medication.

1. As has already been mentioned, there are indications that all or nearly all mature flukes have been killed if a very low egg count after treatment is maintained for at least two weeks. It seems therefore to be improbable that a gradual rise of the count as in type V is caused by flukes that survived medication at the adult stage, and which gradually resume egg production. If that is so, the gradual rise must have been caused by flukes which were immature at the moment of treatment.

2. Types IV and V have been observed in sheep, and considerable numbers of immature flukes have also been found in the livers of sheep from time to time. In the livers of cattle, on the other hand, never more than a few immature flukes have been found. Type V has not been encountered after treatment of these animals, while type IV can not clearly be distinguished, probably because insufficient numbers of immature flukes were present in the livers of these animals.

3. Some observations seem to indicate that it takes about twenty or some more weeks after the last ingestion of encysted metacercariae by the host for all the flukes to reach the stage of full egg production:

a. The egg count of calf 1115 (Table 5) shows no further rise twenty weeks after the last administration of encysted metacercariae. The egg count of sheep 45692 (Table 27) stops rising 22 weeks after purchase, and the same seems to be the case with cow 325452 (Table 7) 16 weeks after purchase.

b. A living immature fluke was found in the liver parenchyma of sheep 1040 (Table 26) as long as 14 weeks after purchase.
Immature worms were not found in animals which were slaughtered more than 18 weeks after purchase.

c. Types IV and V were not observed in sheep longer than 21 weeks after purchase, apart from sheep 1546 after treatment (E) (Table 32), where a rather gradual rise of the egg count might be the result of slow excretion of hexachlorophene after subcutaneous injection of an oily solution of the drug.

In view of the results of the *post-mortem* examinations and the interpretations of the different courses of the egg count after medication, the results of the treatments can be evaluated as follows.

2. Effectiveness of treatments with alkyl tin compounds

A list of the treatments is provided in Table I.

A dose of 10 mg/kg of di-n-butyl tin dilaurate, administered to cow 360413 (Table 4), resulted in a slight reduction in the mean weekly egg count.

The same drug, administered at a rate of 20 mg/kg to cow 360406 (Table 4), resulted in a temporary reduction in the number of fluke eggs in the faeces.

A dose of 30 mg/kg of di-n-butyl tin dilaurate, administered to calf 1115 (Table 5), appears to have caused a moderate reduction in the number of eggs.

If these treatments are compared, it should be realized that calf 1115 had been experimentally infected. Liver fluke infection in this animal had been induced comparatively recently, and liver lesions could not yet be extensive. When the calf was slaughtered, the liver was found to have calcified bile ducts, but the organ was passed as fit for consumption after removal of the affected portions. The liver of cow 360413, on the other hand, showed all the signs of chronic fascioliasis, and the organ was condemned at the slaughterhouse. This was probably the case also with the liver of cow 360406, as this was also an old animal with a chronic infection. It may be possible that the flukes in the liver of calf 1115 were more easily killed by drugs than the parasites in the badly affected livers of the old cows.

Administration of 400 mg/kg of di-n-octyl tin dilaurate to calf 1115 (Table 5) was followed by a drop in the egg count four weeks later. It seems doubtful whether the reduction in the number of eggs after such a long interval was due to the treatment.

A dose of 200 mg/kg of di-n-octyl tin dichloride has been administered to sheep 45695 (Tables 22a and 22b). The egg count showed a reduction after the treatment, and thereafter the number of eggs gradually increased. The fact that the number of eggs began to decrease in the week of treatment suggests that the drug had affected a number of flukes. The low value of the egg count on the day after treatment is probably mainly a result of the diarrhoea, which was observed on that day.

Conclusion

The results suggest that alkyl tin compounds have produced some effect upon mature liver flukes.

3. Effectiveness of treatments with carbon tetrachloride

A list of the treatments with carbon tetrachloride is provided in Table II.

Administration by the oral route

The first dose of 1 ml CCl_4, administered to sheep 479 (Table 23), resulted in a considerable reduction in the number of eggs in the faeces, but this number subsequently increased significantly. Since the animal was dosed 16 weeks after purchase, it is not impossible that immature flukes were present at the time of medication. In that case young worms could have escaped the action of the drug, and have matured afterwards. Nevertheless the count of 45 eggs per gram of faeces nearly three weeks after treatment suggests that the mature flukes had not all been killed.

The second treatment of sheep 479 (Table 23), which was carried out with the same dose about 27 weeks after purchase, has to be considered as having yielded very disappointing results. As the presence of immature flukes was unlikely, the treatment seems to have resulted in no more than a temporary reduction in the number of eggs produced by the flukes.

The administration of 3 ml CCl_4 to cow 2273 (Table 6) appears not to have affected the egg production of the mature flukes. This animal had received higher doses of CCl_4 on previous occasions.

A dose of 4 ml CCl_4, administered to cow 2273 (Table 6, treatment (A)), resulted in a reduction in the mean weekly egg count. Evidently a high proportion of the mature flukes have survived. Another treatment of this animal with the same dose (Table 6, treatment (C)) has hardly influenced the egg count. This also was the case after treatment of cow 327129 (Table 8) with 4 ml CCl_4.

A dose of 5 ml CCl_4, which was given to cow 2273 (Table 6), caused a reduction in the number of eggs excreted with the faeces, but a considerable proportion of the mature flukes apparently survived. The same dose, applied to cow 325452 (Table 7), caused a more marked decrease of the number of eggs, but also in this case the effect on mature flukes was not satisfactory.

Administration by intramuscular injection

A 5 ml dose, which has been administered to cow 327129 (Table 8), was not followed by any reduction in the number of eggs in the faeces.

The first treatment of cow 325452 with 10 ml CCl_4 (Table 7, treatment (A)) did not result in an obvious lowering of the mean faecal egg count. The result of the second

treatment of this animal with the same dose (Table 7, treatment (C)) was also doubtful.

A dose of 20 ml CCl_4, administered to cow 327129 (Table 8), did not seem to have significantly influenced the numbers of eggs in the faeces.

A dose of 30 ml CCl_4, administered to cow 327129 (Table 8) seems to have resulted only in a slight reduction in the mean egg count.

Conclusion

The first treatment of a sheep with an oral dose of 1 ml CCl_4 yielded fairly good results against mature liver flukes, but the efficacy of a second treatment of this animal with the same dose was unsatisfactory.

Oral doses of up to 5 ml CCl_4 did not yield satisfactory results against mature liver flukes in cows.

Intramuscular injection of doses up to 30 ml CCl_4, administered to cows, resulted in no more than a slight reduction in the number of fluke eggs in the faeces.

4. Effectiveness of treatments with "Vitan"

All treatments, and the results thereof, have been mentioned in Table 9.

A dose of 8 ml was administered to calf 4139 (treatment (D)) and calf 7642 (treatment (H)). Treatment of calf 4139 resulted in a moderate reduction in the number of eggs in the faeces. In the case of calf 7642 the output of fluke eggs with the faeces was very irregular, and consequently it was not possible to obtain a clear-cut picture of the efficacy of the treatment. However, it is clear, that many mature flukes have survived.

Ox 5111 received a dose of 12 ml (treatment (E)), and this treatment resulted in an unsatisfactory reduction in the number of eggs in the faeces.

A dose of 16 ml was administered to ox 5111 (treatment (F)), and the animal received the same dose eight days later (treatment (G)). These treatments produced no discernable effect.

A dose of 20 ml was given three times to cow 1864. Although the egg count was already very low before treatment, eggs were still found after treatment (A). Following treatments (B) and (C) very small numbers of eggs were occasionally present in the faeces. As the egg counts were very low, it is not possible to estimate the degree of efficacy of the treatments of this animal. Treatment (J) of cow 8902 with a dose of 20 ml, resulted only in a temporary reduction in the number of eggs in the faeces.

Conclusion

Treatments with "Vitan" in doses as advised by the manufacturer have given unsatisfactory results against mature *Fasciola hepatica* in cattle.

5. Effectiveness of treatments with symmetric difluorotetrachloroethane, Freon 112

A dose of 50 g Freon 112, administered to cow 217561 (Table 18), seems to have caused some reduction in the number of eggs in the faeces.

The result of treatment of cow 326539 with a dose of 200 g (Table 18) was not much better than that obtained with the lower dose.

Conclusion

The effectiveness of the drug against mature flukes in chronically infected cows appeared to be disappointing.

6. Effectiveness of treatments of cattle with *2,2'*-methylenebis (3,4,6-trichlorophenol), hexachlorophene, G-11

The treatments of cattle with hexachlorophene are listed in Table III.

Administration of G-11 in watery suspension by the oral route

A dose of 10 mg/kg, administered to cow 301660 (Table 12), very efficiently reduced the number of eggs in the faeces.

The effect of administration of 14 mg/kg to cow 662205 (Table 14) was also very good. Only occasionally very small numbers of eggs were present in the faeces after the second week following treatment. Probably all, or nearly all mature flukes were killed.

Very good or excellent results have been obtained by administering a dose of 15 mg/kg to 13 cattle. The numbers of living mature flukes per animal, found on autopsy, were the following:

0: 7 animals (9511: Table 10; 301694: Table 12; 208211, 368640: Table 13; 452133, 779763, 321437: Table 14)
1: 4 animals (5104: Table 10; 301660: Table 12; 209810, 369304: Table 14)
2: 1 animal (208247: Table 13)
7: 1 animal (301635: Table 12).

The treatment of cow 301635 was not entirely satisfactory. In the other animals, however, the degree of reduction in egg count, and the results of *post-mortem* examination suggest that all or nearly all mature flukes were killed.

Occasionally *Fasciola* eggs have been found in the faeces after medication of animals which seemed to be freed from living mature flukes. Although it is possible that some flukes remained undetected in the bile ducts, during *post-mortem* examination, the presence of those eggs does not necessarily mean that living mature flukes escaped detection. Dead mature flukes, which often contained great numbers of eggs, have

been found in the bile ducts of all but one animal. Decomposition of dead worms in the bile ducts and intestine will liberate their eggs, and intact dead worms in faecal samples, collected for examination, may be destroyed in the stirring process. There are still other facts which may account for the occasional presence of eggs in the faeces of animals which do no longer harbour living mature flukes. Pus was present in the bile ducts of several animals, and more or less complete obstruction was often caused by solid or paste-like substances. Fluke eggs have sometimes been found on examination of these materials. Obviously eggs may be trapped in such bile ducts for a considerable length of time, and they may eventually pass to the intestine.

Immature flukes have been found only on one occasion, *i.e.* in the liver parenchyma of cow 301660, where the five young parasites were alive.

A dose of 20 mg/kg yielded excellent results in seven animals. In the livers of two animals (208489 and 779768: Table 15), which were slaughtered four weeks after treatment, no living mature flukes have been found. The egg counts following treatment of the five remaining cattle (1531, 1556, 2927: Table 15; 734, 26737: Table 16) suggest that medication has also been extremely effective in these cases.

Administration of G-11, solubilized in water with Tween 80, by the oral route

Only one treatment was carried out with a dose of 5 mg/kg of hexachlorophene: cow 305786 (Table 10). The course of the mean weekly egg count conformed to Type III. After the initial drop in the first week following treatment, a moderate ultimate reduction in the number of eggs has been obtained.

Administration of an oily solution of G-11 by the oral route

A dose of 10 mg/kg of hexachlorophene, administered to cow 301694 (Table 12), resulted in a very satisfactory reduction in numbers of eggs, although the eggs did not completely disappear during three weeks following treatment. Probably one or a few mature flukes remained alive.

Excellent results were obtained by medication of cow 208497 (Table 13) with a dose of 14 mg/kg, and of cow 31568 (Table 13) with a dose of 15 mg/kg of hexachlorophene. Only one living mature fluke was found in each of these animals.

Two out of four immature flukes found in the bile ducts of cow 208497 were dead. It seems therefore, that this treatment was partially effective against immature flukes which were present in the bile ducts.

Administration of an oily solution of G-11 by subcutaneous injection

The course of the faecal egg count following both treatments with hexachlorophene at a dose rate of 5 mg/kg (cows 5104 and 9511: Table 10) conformed to Type III. Considering the ultimate reduction in number of eggs, some effect has been obtained by treatment of cow 5104, whereas a considerable number of mature flukes seems to have been killed by medication of cow 9511.

A dose of 10 mg/kg of hexachlorophene, administered to cows 301635 and 207551 (Table 12), resulted in a very marked reduction in quantity of eggs. Complete eradication of the mature flukes was not obtained in cow 301635, and probably not in cow 207551.

Excellent results have been obtained by treating cow 208084 (Table 13) with hexachlorophene at a dose rate of 15 mg/kg, as only dead flukes were found on autopsy seven weeks after medication.

Administration of an alcoholic solution of G-11 by subcutaneous injection

Treatments with hexachlorophene at dose rates of 39 and 40 mg/kg, respectively (calf 4139 and ox 5111: Table 16), were apparently completely successful.

Conclusion

Different modes of application provided comparable results at a given dose rate.

A dose of 5 mg/kg of G-11 resulted in a slight or moderate reduction in the number of mature flukes.

A dose of 10 mg/kg of G-11 very efficiently reduced the number of eggs in the faeces, but apparently a small number of mature flukes remained alive.

Hexachlorophene at a dose rate of 15 mg/kg showed excellent activity against mature flukes. All or nearly all mature flukes were killed. Only in one case was the efficacy of this compound not entirely satisfactory.

No indications were found that flukes survived after treatment with G-11 at dosage rates of 20 mg/kg and higher.

7. Effectiveness of treatments of cattle by administration of a combination of hexachlorophene and other compounds by the oral route

Treatments were carried out with hexachlorophene at the dose rate of 5 mg/kg in combination with one of the following compounds: carbon tetrachloride, hexachloroethane, sodium pentachlorophenate, dichlorophene and bithionol. These combined treatments are listed in Table III, and the relevant data are referred to in Tables 10 and 11.

A dose of 5 mg/kg of G-11 and 2 ml carbon tetrachloride, administered to cow 217561 (Table 10), resulted in no more than a slight reduction in the number of eggs in the faeces after an initial period in which egg production of the worms had been temporarily reduced (Type III).

A similar course of the mean weekly egg count was observed after treatment of cow 326539 (Table 10) with 5 mg/kg of G-11 and 15 g hexachloroethane. The ultimate reduction in egg count was not much better than in the treatment of cow 217561.

A very marked reduction in the number of eggs in the faeces was observed after medication of cow 305786 (Table 10) with a combination of 5 mg/kg of G-11 and 50 mg/kg of sodium pentachlorophenate. Both the *post-mortem* findings and the degree of reduction in egg count suggested that a high proportion of the mature flukes were killed by the treatment.

Hexachlorophene, combined with dichlorophene, was administered to three cows. A moderate reduction in egg count was obtained by treatment with 5 mg/kg of G-11 and 40 mg/kg of G-4 (cow 306493: Table 11). A dose of 5 mg/kg of G-11 and 7 mg/kg of G-4 (cow 460288: Table 11) caused a marked reduction in the mean egg count. The treatment of cow 361084 (Table 11) with 5 mg/kg of G-11 and 0.7 mg/kg of G-4 seemed to result only in a slight reduction in the number of eggs.

Administration of 5 mg/kg of G-11 and 0.4 mg/kg of bithionol to cow 429951 (Table 11) has resulted in a moderate reduction in the egg count.

Conclusion

The combined treatments failed to yield very satisfactory results. Treatments with combinations of G-11 and either sodium pentachlorophenate or dichlorophene, however, provided results which seem to warrant further trials.

8. Effectiveness of treatments of sheep with 2,2'-methylenebis (3,4,6-trichlorophenol), hexachlorophene, G-11

The treatments of sheep with hexachlorophene are listed in Table IV.

Administration of G-11 in watery suspension by the oral route

A dose of 5 mg/kg gave excellent results in three sheep (1596, 1597, and 1598: Table 24), while results in two other animals (1117 and 1133: Table 25) were disappointing, as no more than a temporary suppression of egg production occurred. The course of the egg count in sheep 1598 conformed to Type V, and a living immature fluke was found in a bile duct. These facts suggest that the treatment of this animal was ineffective against immature flukes.

A dose of 10 mg/kg showed very good efficiency in one sheep (1595: Table 25), poor efficiency in another sheep (1599: Table 25), and probably had a good effect in the third animal (P: Table 25).

It thus appears that this mode of application at both dose rates did not give consistent results. As treatment of some of the animals had given results which were far from satisfactory, it was considered that consistent results could not be expected from a slight increase of the dosage rate.

Administration of an oily solution of G-11 by the oral route

In both animals (1083 and 1181: Table 24) which received 5 mg/kg of hexachloro-

phene, the course of the egg count corresponded to Type V, and therefore it was considered that the treatments had been very effective against mature flukes but not against immature worms.

The effect of treatment of the sheep 1117 and 1133 (Table 25) with 10 mg/kg appeared to be somewhat less satisfactory against mature and nearly mature flukes than that of the treatments with the lower dosage, and immature flukes seem to have not been killed.

A dose of 15 mg/kg, which was administered to nine sheep, gave excellent results against mature flukes in seven animals (45681, 45683, 45685, 45689, 1040, 1031: Table 26; 1153: Table 27). The course of the egg count following treatment of sheep 1040 conformed to Type V, and a living immature fluke was found in the liver parenchyma after slaughter of the animal ten weeks after treatment. These facts indicate that young flukes were present, and survived, when the animal was treated, and the writer believes that the five mature flukes found on autopsy were not yet mature at the moment of treatment. The course of the egg count of sheep 1153 also conformed to Type V, and a number of immature flukes probably survived the treatment. The dose of 15 mg/kg was fully effective in sheep 45692 (Table 27) against mature flukes, whereas a number of immature flukes also escaped the action of the drug. Only a temporary suppression of egg production was observed after treatment of sheep 1546 (Table 32). In this case treatment was apparently quite ineffective.

Consistent results have not been obtained against mature flukes with this mode of application, using doses of up to 15 mg/kg, and indications have been found that immature flukes survived.

Administration of a solution of G-11 in propylene glycol by the oral route

A dose of 12 mg/kg of G-11 did not seem to kill mature flukes in sheep 1117 (Table 25).

Treatment with a dose of 15 mg/kg, applied to nine sheep, gave excellent results against mature flukes in six sheep (1083, 1181: Table 24; 1153: Table 27; 45682, 45684, 45691: Table 28). In the case of sheep 45691 eggs were found in the faeces four and five weeks after treatment, and six living mature flukes were found on autopsy six weeks after treatment. These findings were probably due to the presence of flukes which had survived treatment at the immature stage. Medication of the remaining sheep (1599, 1133: Table 25; 1546: Table 32) did not seem to cause any permanent injury to mature flukes.

A dose of 23 mg/kg had no effect on the flukes in sheep 1117 (Table 25), and a dose of 30 mg/kg, administered to sheep 1546 (Table 32) probably did not result in death of all mature flukes; a number of immature flukes also appears to have survived.

Administration of G-11, solubilized in water with Tween 80, by the oral route

All 21 treatments resulted in death of all or nearly all mature flukes. The *post-*

mortem findings in nine of the sheep (40820, 40821, 40822, 40825: Table 30; 40815, 40816, 40818, 40819, 40830: Table 31), which received 30 mg/kg of hexachlorophene, present strong evidence that this treatment was lethal for all immature flukes which were present in the liver parenchyma and in the bile ducts.

Subcutaneous injection of a solution of G-11 in olive oil

A dose of 5 mg/kg of G-11 was not completely effective against the mature flukes of sheep 1545 (Table 24).

A dose of 15 mg/kg, administered to eight sheep, resulted in excellent results against mature flukes in six animals (1599, 1117, 1133: Table 25; 1547, 45686, 45694: Table 27). Two dead young flukes were found in the liver parenchyma of sheep 45686. The course of the egg count following treatment of sheep 45694 conformed to Type V; possibly the two mature flukes found on *post-mortem* examination were parasites which had survived treatment at the immature stage. Medication of sheep 45690 (Table 27), and of sheep 1546 (Table 32) apparently did not result in death of all mature flukes.

Subcutaneous injection of a solution of G-11 in Tween 80

Two sheep were treated with a dose of 15 mg/kg of G-11. Excellent results against mature flukes were obtained in sheep 45692 (Table 27); the results were not completely satisfactory in sheep 45693 (Table 27).

Conclusion

Treatment of sheep with G-11 yielded inconsistent results. While in some instances a dose of 5 mg/kg was sufficient to kill practically all mature flukes, higher doses (up to 30 mg/kg) sometimes had little or no effect. It appeared that the flukes in a few individual sheep (1117, 1133, 1546, and 1599) were relatively resistant to the action of the drug, and that the mode of administration is an important factor which determines efficacy.

The only treatment which yielded uniformly excellent results was the administration by the oral route of G-11 solubilized in water with Tween 80 at dose rates of about 20–30 mg/kg of G-11. This treatment seemed also to be very effective against immature flukes.

9. Effectiveness of treatments of sheep with hexachlorophene-piperazine complex

The treatments are listed in Table V.

Administration of a watery suspension by the oral route

Treatment of sheep 1546 (Table 32, treatment (A)) with a dose of 15 mg/kg resulted

in a course of the mean egg count conforming to Type III. The ultimate reduction in egg count was unimportant.

Subcutaneous injection of a suspension of the drug in olive oil

Excellent activity against mature flukes was observed after administration of 30 mg/kg to sheep 45687 (Table 33). Aluminum monostearate was added with the purpose of trying to delay absorption of the complex compound. The course of the egg count conformed to Type VII, suggesting that a depot of hexachlorophene-piperazine complex remained under the skin for several weeks, so that a number of flukes was not killed immediately but only after a long delay. Other explanations, however, seem also possible.

The administration of about 35 mg/kg to sheep 1546 (Table 32, treatment (F)) did not result in any reduction in the number of eggs. Apparently the flukes had not been affected.

Medication of sheep 45695 (Table 33) with hexachlorophene-piperazine complex at the dose rate of 40 mg/kg resulted in a satisfactory reduction in the output of eggs.

A dose of 50 mg/kg, administered to sheep 45688 (Table 33), appears to have been completely successful in killing mature flukes.

Conclusion

A dose of 15 mg/kg, administered orally to a sheep, was ineffective. Administration of higher doses by subcutaneous injection yielded inconsistent results.

10. Effectiveness of treatments of cattle with 2,2'-thiobis (3,4,6-trichlorophenol), G-11S

The three treatments, which were carried out by administering a watery suspension of G-11S by the oral route, have been mentioned in Tables 17a and 17b.

In the week before medication only three daily egg counts were carried out. In order to obtain a clearer picture of the degree of infestation before treatment, the daily egg counts in the week of treatment have also been given.

A dose of 5 mg/kg, administered to heifer 1744, resulted in some reduction in egg count.

A dose of 10 mg/kg (heifer 1690) has yielded much better results. The number of eggs, passed in the faeces, was satisfactorily reduced, but probably not all mature flukes were killed.

The egg counts following medication of heifer 1738 with a dose of 15 mg/kg suggest, that the treatment has been fully effective against mature flukes.

Conclusion

The results of treatments with G-11S suggest a relationship between efficacy and dosage. The treatment with a dose of 15 mg/kg yielded excellent results.

11. Effectiveness of treatments of cattle with 1,4-di(trichloromethyl)benzene ("Hetol")

The treatments are listed in Table VI.

Cow 326686 (Table 19) received 80 g "Hetol", the maximum dose recommended by the manufacturer. The dose rate was 99 mg/kg of 1,4-di(trichloromethyl)benzene. The egg count after treatment showed a course conforming to Type III, and no ultimate reduction in the number of eggs per gram of faeces was observed. It seems that ovulation of the worms was temporarily suppressed, and that no flukes were killed.

A dose of 136 mg/kg of 1,4-di(trichloromethyl)benzene was given to two cows. The treatment of cow 369941 (Table 19) resulted in a moderate reduction in egg count, and in the case of cow 326539 (Table 19), in no obvious ultimate decrease in the number of eggs.

Seven cows received 1,4-di(trichloromethyl)benzene at a dose rate of 150 mg/kg. The pre-treatment egg counts of three of these animals (326539: Table 19; 217561, 325452: Table 20) were very low; this suggested the presence of very small numbers of mature flukes. Two living flukes were found in the livers of each of these three cows when the animals were slaughtered after treatment. These data suggest that the treatments did not yield very satisfactory results. The data concerning the treatments of the four other cows, which also received a dose of 150 mg/kg of 1,4-di(trichloromethyl) benzene, support this view. The result of medication of cow 420219 (Table 20) was fairly good considering both egg counts and *post-mortem* findings, but the results in cows 326686, 369941 (Table 19), and 327129 (Table 20) have been disappointing.

Very good or excellent results were obtained by administering a dose of 225 mg/kg of 1,4-di(trichloromethyl)benzene to the cows K88033, 241792, 17397 and 364024 (Table 21). Only the result of treatment of cow 712068 was not very satisfactory.

The egg counts indicate that flukes, which were not killed by treatment, produced very few or no eggs in the first two or three weeks after medication. The apparent suppression of egg production by surviving flukes appears to persist for at least about three to five weeks. Several of the living adult flukes, found in the livers of cows 326686, 369941 and 327129 six weeks after the last treatment, contained few or no eggs. These facts show that egg counts, performed within a period of several weeks following treatment with "Hetol", are of very little value for estimating the efficacy of medication.

Conclusion

1,4-Di(trichloromethyl)benzene at a dose rate of 225 mg/kg showed very good activity against mature *Fasciola hepatica* in cattle. Doses of 150 mg/kg and lower did not yield satisfactory results.

12. Effectiveness of treatments of sheep with 1,4-di(trichloromethyl) benzene ("Hetol")

The six treatments, mentioned in Table 34, apparently yielded excellent results.

Conclusion

It appears that a dose of 160 mg/kg of 1,4-di(trichloromethyl)benzene is practically fully effective against mature flukes in sheep.

IX OBSERVATIONS ON THE TOXICITY OF THE EMPLOYED FASCIOLICIDES

1. Alkyl tin compounds

Sheep 45695 (Table 22) suffered from diarrhoea the day following treatment with 200 mg/kg of di-n-octyl tin dichloride. The other treatments with alkyl tin compounds have been perfectly tolerated.

2. Carbon tetrachloride in cattle

Administration of 5 ml CCl_4 by the oral route to cow 325452 (Table 7) resulted in diminished appetite and inactivity on the first two days following treatment. However, this animal did not exhibit signs of intoxication after the intramuscular injections of 10 ml CCl_4.

Apart from the effects observed in cow 325452 after an oral dose of 5 ml CCl_4, symptoms of intoxication were not observed although doses as high as 20 or 30 ml have been given by intramuscular injection. On two occasions a swelling at the site of injection was observed: after treatment (A) of cow 325452 (Table 7), and treatment (A) of cow 327129 (Table 8). In the former case a single injection of 20 ml of the mixture of carbon tetrachloride and liquid paraffin was given, in the latter case the dose was divided into two injections, each of 30 ml of the mixture. The swellings developed the day after injection, and disappeared gradually in the course of about a month. In these cases the quantity given by a single injection was probably too large, for swellings did not develop when injections of 15 ml or less of the mixture were given.

3. "Vitan" in cattle

The day following the administration of 20 ml "Vitan", cow 8902 (Table 9) was inactive, and the animal showed a diminished appetite. Symptoms of intolerance were not observed following the remaining eight treatments applied to four animals (Table 9).

4. Freon 112 in cattle

The two treatments (Table 18) did not result in untoward effects.

5. Hexachlorophene in cattle

a. The tolerance of cattle towards hexachlorophene

With dosages up to 15 mg/kg symptoms of general intoxication were not exhibited by the experimental animals. Only two signs of local irritation should be mentioned: diarrhoea after medication by the oral route, and considerable tissue reactions after subcutaneous injection of an oily or alcoholic solution of the drug. Diarrhoea was observed only after treatment (D) of cow 408247 (Table 13) and after treatment (D) of cow 662205 (Table 14), and it occurred only during a few hours the day after treatment. After subcutaneous inoculation an extended swelling developed around the site of injection. These swellings persisted for at least several weeks, and did not always disappear completely. A calf, which received subcutaneously a comparable amount of pure olive oil (sterilised in the same way as the solution of G-11 in olive oil), did not develop a swelling at the site of injection. Less severe tissue reactions may possibly occur if the dose were divided into more injections at different sites of the body. This was not tried, because it was found that the drug was at least as effective when administered orally. Treatment by subcutaneous injection was discontinued after its results had been observed in five animals.

Oral administration of an aqueous suspension of the drug, in doses up to about 15 mg/kg, was used in the field on a larger scale. Since January 1959 about 1,200 treatments of cattle of different ages have been carried out in different seasons. In the first two years the dose was about 12.5 mg/kg of G-11; as the body weights had to be estimated, the real dose was probably between 10 and 15 mg/kg. In the year 1961 the estimated dose rate was regularly increased from 12.5 to 15 mg/kg, and a number of animals will have received more than 15 mg/kg of G-11. Cows expected to calve within a few days, and animals which had calved less than a week before, received somewhat less than 15 mg/kg. Among the treated cattle were animals (mainly calves and yearlings) that suffered from clinical liver fluke disease. These animals, which were underdeveloped and often emaciated, sometimes received an initial dose of about 10 mg/kg. For most of the treatments hexachlorophene tablets were employed, as mentioned under Table 14. All cattle of four farms received six treatments over a period of two years. Among these animals were ten calves which received the treatment ten days after medication with a mixture of hexachloroethane and phenothiazine.

The treatments of cattle in the field were perfectly tolerated. Diarrhoea during the first day after treatment was rather common, and cattle heavily infected with liver flukes seemed sometimes to show a slightly diminished appetite on that day. Only one case of some degree of intolerance has been encountered: a two-year-old animal, which was emaciated and much too small for its age, and which showed a count of 400 *Fasciola* eggs per gram of faeces, refused all food the day after treatment. Thereafter the animal did not exhibit any abnormality.

With doses higher than 15 mg/kg obvious signs of intoxication were sometimes observed.

Apart from the seven animals mentioned in the Tables 15 and 16, eight other animals were tested for their tolerance towards an oral dose of 20 mg G-11/kg. Eight cows were given this dose in the advanced stage of gestation, while six received a second dose one to eight days after calving, the interval between both treatments for each animal being no shorter than two weeks. The symptoms of intoxication observed after an oral dose of 20 mg G-11/kg are summarized in the tables 35 and 36. Only cow 1531 showed clear symptoms. The results suggest that cattle treated within a short period before or after parturition sometimes are more susceptible to the toxic action of the drug than other animals. Generally, however, a dose rate of 20 mg/kg is perfectly tolerated at these stages.

More or less alarming symptoms of intoxication were observed with some of the animals, which were subjected to tests on the tolerance of cattle towards higher doses of hexachlorophene.

A dose of 30 mg G-11/kg body weight was administered by stomach tube to fifteen animals of different ages. The results are summarized in Table 35. Three animals did not exhibit any symptoms, and two other animals only suffered from diarrhoea during the first day after treatment. The symptoms exhibited by the other animals generally persisted for only one or two days, and all animals recovered completely within a week. Apart from increased respiratory rate, which was very pronounced in the yearling 1526 on the first and second day after treatment, the most remarkable symptom was inactivity, as characterized by standing very quietly, often with the neck stretched and with the head somewhat low. This was accompanied by saliva flowing out of the mouth in the case of calf 1521 the second day after treatment.

One calf was treated with a dose of 39 mg G-11/kg, and three animals received 40 mg G-11/kg (Table 35). A watery suspension of the drug was administered by stomach tube to calf 1217; the three other animals received by subcutaneous injection at one or both sides of the neck a solution of hexachlorophene in ethyl alcohol. Calf 4139, ox 5111 and cow 8902 were carrying *F. hepatica*, while fluke eggs were not found in the faeces of calf 1217 prior to treatment. Calf 4139 often lay down the third day after treatment, and ox 5111 ate somewhat less than normal that same day. Calf 1217 also was not seriously affected, but cow 8902 became severely ill a few days after treatment. The animal began to lose appetite on the third day after treatment. The next day the animal displayed elevated temperature, and a higher pulse rate. Oedema developed at both sides of the neck at the site of injection. At a later stage the whole neck was swollen. On the 6th day after medication the animal died after having shown a rectal temperature of over 42°C, abundant sweating and a high respiratory rate. During the last days the animal kept the head low, saliva flowing out of the mouth. Subcutaneous injections of a solution of calcium borogluconate on the 4th and 5th day, and an intravenous injection of a solution of calcium chloride on the

6th day after treatment with G-11, were apparently not beneficial. Autopsy did not reveal specific abnormalities. The liver was swollen, and the kidneys were somewhat enlarged. There was no centrolobular necrosis of the liver.

The other animals, which were injected subcutaneously with an alcoholic solution of hexachlorophene, also developed swellings at the sites of injection.

b. Influence of hexachlorophene medication on the milk production

As a drop in milk production after medication is a sensitive indication of toxicity of drugs, the daily milk yield of fourteen cows was determined before and after administration of hexachlorophene at different dosage rates. The results are given in the Tables 37 and 38. The four treatments with 30 mg G-11/kg are also mentioned in Table 35, where other symptoms of intoxication are reported.

It may be concluded that a dose of 15 mg G-11/kg body weight did not materially affect milk production in eight of the nine cows, while one (cow 9511) showed only a slightly diminished milk yield the 2nd and 3rd day after medication, possibly as a result of the treatment. The cow treated with 20 mg G-11/kg, and the four animals which received 30 mg/kg, showed a drop on the 2nd, or the 2nd and the 3rd day after medication only. The drop in cow 301694 could be attributed to mastitis.

c. Hexachlorophene medication and semen production

A watery suspension of hexachlorophene, prepared by the addition of sodium lauryl sulphate, was administered by stomach tube to a young steer, which weighed 612 kg. The dose rate was 15 mg G-11/kg. Prior to treatment two ejaculates, one after another, were collected for examination twice a week in the course of five weeks. Determination of the quantity of semen, of spermatozoon mobility, and of wave-motion, as well as the spermatozoa counts, were carried out under the direction of Mr. H. G. VAN WAVEREN at the Centraal Diergeneeskundig Instituut (Central Veterinary Institute) at Rotterdam. The morphology of the cells was studied under the direction of Prof. Dr. F. C. VAN DER KAAY, director of the Kliniek voor Veterinaire Verloskunde en Gynaecologie (Clinic for Veterinary Obstetrics and Gynecology) of the State University at Utrecht.

One ejaculate was collected daily on each of the first four days after treatment, and the material was examined in the same way. Since it has been suggested that an effect on spermatogenesis may come to light only after 40–45 days, semen was also examined twice a week in the period covering four to nine weeks after treatment.

It was concluded that a detrimental effect of the treatment on either quantity or morphological quality of the semen could not be established.

d. Influence of hexachlorophene medication on animal products

GREGG & ZOPF (1951) found that hexachlorophene is freely soluble in corn oil, olive oil, and cottonseed oil. Although the drug is probably also readily soluble in

animal fats, there are indications that accumulation in the body fat, or in the fat fraction of the milk of medicated animals, is not very likely to occur.

The faecal egg counts indicate that G-11 acts as rapidly on the flukes, and to a similar degree, after subcutaneous injection of an oily solution of the drug as after administration by the oral route of a watery suspension. The course of the egg count following medication with dose rates lower than 15 mg G-11/kg is also similar after both modes of administration: after the initial drop, the egg count rises as rapidly, and the rise is completed after a similar interval.

As the egg count is reduced to its lowest level within a few days, it may be concluded that hexachlorophene becomes available very rapidly, and in considerable quantity, from the oily solution deposited under the skin. The oil is not quickly resorbed, as appeared after slaughter of cow 207551, when a considerable amount of oil was found to be present under the skin 23 days after injection. The egg counts, however, indicated that the effect of hexachlorophene on the flukes had disappeared within about two weeks after injection.

These facts suggest that G-11 is released rapidly from the oil deposit under the skin, and that the store of G-11 is depleted before the oil has been resorbed completely. It seems therefore likely that the affinity of hexachlorophene for the surrounding body fluids and tissues is greater than for the olive oil, and probably the affinity for animal fats is not much greater. It would therefore seem unlikely that hexachlorophene will accumulate in the body fat or in the butter.

Experiments on the excretion of hexachlorophene, after administration by the oral route of a watery suspension of the drug to lactating cows, were carried out in cooperation with the Rijks Instituut voor de Volksgezondheid (National Institute of Public Health) at Utrecht. The preliminary results indicated that more than 50% of the administered quantity was excreted unchanged in the faeces within a few days. Only minute amounts could be detected in the urine during the same period. Any evidence for the presence of G-11 in the milk could not be obtained by the analytical methods used.

Cow 207551 had to be slaughtered, because of perforation of the oesophagus, two days following administration by stomach tube of hexachlorophene (15 mg/kg) as a watery suspension. Cooking and frying of meat of this animal, carried out at the municipal slaughterhouse at Rotterdam, did not reveal an abnormal flavour.

Milk of treated animals could never be distinguished from that of unmedicated cows.

6. Hexachlorophene in sheep

The following treatments of experimental sheep with hexachlorophene have been carried out:

5 mg/kg: 8 treatments
about 10 mg/kg: 6 treatments
15 mg/kg: 28 treatments
about 20 mg/kg: 11 treatments
30 mg/kg: 14 treatments

Following 61 of these 67 treatments no other reaction was observed apart from a slight inactivity on the first day after treatment in 8 sheep, which had received G-11, solubilized in water, at the dose rate of 20 mg/kg, by the oral route, and diarrhoea in two animals the second day after administration of 30 mg/kg of G-11 in the solubilized form. A softening of the faeces occurred on a few occasions during the first one or two days following treatment by the oral route.

The remaining six cases have to be discussed in some detail.

1. It has already been mentioned in Table 24, that sheep 1545 died four weeks after treatment. The dose was only 5 mg G-11/kg, and the animal had not shown signs of acute intoxication. These facts, as well as the long interval between medication and death, suggest that the cause of the sudden death was not hexachlorophene poisoning.

2. Symptoms of intoxication have been observed after oral administration of G-11 (15 mg/kg) in oily solution to sheep 1153 (Table 27). This animal suffered from diarrhoea on the first two days after treatment. The appetite began to diminish on the 2nd day after treatment, and the animal showed inactivity at the same time. From the 4th day on the animal was lying most of the time. Gradual recovery began on the 9th day after treatment.

The pre-treatment egg counts suggest that this animal had a high worm burden when it was treated. When the sheep was slaughtered 14 weeks later, the liver was found to be extensively cirrhotic. The animal had probably been sensitive to the toxic action of the drug because of the diseased condition of the liver, which was due to the liver fluke infection.

In the four following cases, hexachlorophene medication was followed by severe disease or death. *Post-mortem* examination of these animals was carried out by Mr. J. P. W. M. AKKERMANS.

3. Sheep 1031 (Table 26) suffered from diarrhoea on the first and second day after medication. One day later diminished appetite and inactivity became apparent, and from the 4th day after treatment the animal was clearly ill. The 7th day after treatment the sheep collapsed, and the animal was slaughtered.

The liver showed hypertrophy of the bile ducts, and rather extensive areas of the liver parenchyma showed degenerative changes and beginning cirrhosis. There was also a chronic perihepatitis, with an adjoining chronic peritonitis. The spleen had a normal appearance. There was no enteritis. One of the kidneys showed a contraction with formation of connective tissue; the other kidney was normal. The lungs were pale and emphysematous, and contained many nodules which appeared to have been caused by lungworms (*Muellerius capillaris*).

4. Sheep 40820 (Table 30), which suffered from diarrhoea the first two days following treatment, had to be slaughtered five days after medication because the animal unexpectedly collapsed.

The liver was severely cirrhosed and affected with pathological fatty change and hyperplasia. The lungs, the kidneys, and the spleen appeared normal. The uterus contained two well developed lambs.

5. Sheep 40823 died within 24 hours after administration of 30 mg G-11 per kg body weight. The drug, which had been solubilized in water with Tween 80 (the fluid was preserved by the addition of orthooxyquinolinesulphate), was administered into the oesophagus by means of an oesophageal drenching apparatus.

The liver was extremely cirrhotic, and the organ showed hypertrophy of the bile ducts. Perihepatitis was also present. The spleen was swollen, and petechiae were present under the capsule. The lungs were oedematous. Fluid was present in the pericardial cavity.

Although the animal was heavily infected with *Fasciola hepatica*, egg counts and worm counts have not been presented in the tables. As the sheep had died at night, it was impossible to determine how many of the worms had been alive when the host died.

6. Sheep 40824 also died within 24 hours after receiving a similar treatment to sheep 40823.

The abdominal cavity contained a large volume of yellowish brown transudate. The liver was nearly as badly affected as that of sheep 40823: it showed a very pronounced cirrhosis and fatty degeneration. Other pathological changes were: petechiae under the capsule of the spleen, catarrhal pneumonia, fluid in the pericardial cavity, and dilatation of the heart. The animal appeared to have been pregnant, and the one lamb present was considered to have died a few days earlier than the mother, and thus before medication.

As in the case of sheep 40823, the bile ducts were not examined for flukes, since sheep 40824 had also died at night.

It is remarkable that the pre-treatment egg counts of the five sheep which showed intolerance towards hexachlorophene, belong to the highest counts which have ever been recorded in our experiments. The three last mean weekly counts (*Fasciola* eggs per gram of faeces) before treatment were respectively as follows:

Sheep 1153:	(2640),	(3000),	1628
Sheep 1031:	1723,	1705,	2182
Sheep 40820:	3380,	3977,	3142
Sheep 40823:	2743,	2963,	4032
Sheep 40824:	4328,	5314,	2907

The egg count on the day of treatment of sheep 40824 was 13625 eggs per gram of faeces.

These egg counts suggest that the extensive damage to the livers of these animals

had been caused by very high burdens of liver flukes. The livers of the sheep 40820, 40823, and 40824 were much more severely affected than those of any other of the experimental sheep, and it seemed very doubtful whether these three animals would have survived without treatment.

Tissue reactions after subcutaneous injection of an oily solution of hexachlorophene were less severe than in cattle. After application of this treatment in nine sheep, an abscess and degeneration of muscular tissue at the site of injection resulted in one animal. The eight other animals did not develop any swelling at the site of injection. In three cases it was found that the flesh at the site of injection was dark-red when the animals were slaughtered six weeks after the treatment. This was also the case with sheep 1545, which died four weeks after the injection. The flesh at the site of injection was unaffected in another sheep, which was slaughtered three weeks after the treatment, and abnormalities were not reported when the remaining three sheep were slaughtered 4, 5, and 26 weeks respectively after the injection.

Sheep 1545 received 5 mg G-11/kg; the eight other animals 15 mg/kg. The whole dose was always administered by a single injection at the inner side of a thigh. The injected volume ranged from 5.9 to 7.8 ml for the animals which received 15 mg G-11/kg. There was no correlation between the quantity of injected oily solution and the degree of tissue damage.

Two sheep received hexachlorophene (15 mg/kg) dissolved in Tween 80, by subcutaneous injection (Table 27). Within 24 hours after the injection at the inner side of the thigh, a swelling began to develop, which rapidly extended over the upper leg and further downwards, and in one animal also, over the adjoining part of the abdomen. Four days after the injection the whole leg was considerably swollen in both animals. The swellings gradually began to shrink during the following days, and by the 16th day after treatment only a thickened ridge was left at the site of injection. This ridge was still present three weeks after treatment in one animal. Apparently Tween 80 is an unsuitable vehicle for subcutaneous injection.

The treatment of sheep against *Fasciola hepatica* by oral administration of hexachlorophene has also been studied in the field. The following treatments were carried out in addition to those referred to above:

about 12.5–15 mg/kg: 28 treatments
about 20 mg/kg: 36 treatments
about 25 mg/kg: 9 treatments
about 30 mg/kg: 42 treatments

As the body weights had to be estimated, several animals received more than 30 mg/kg. Animals of different ages, including lambs of only 12 weeks old, and parturient ewes, were treated, also with the highest dose rate. It should be stressed, however, that none of the animals treated in practice suffered from severe fascioliasis.

The doses up to 15 mg/kg were either given as a solution of G-11 in propylene glycol or as a suspension obtained by soaking hexachlorophene tablets in water. For the

higher dose rates hexachlorophene, solubilized in water with Tween 80, was employed. In nearly all cases this fluid preparation contained orthooxyquinoline sulphate as a preservative.

One lamb accidentally received a dose considerably higher than 30 mg/kg (probably more than 60 mg/kg). According to the owner, this animal was very inactive the day following treatment, and for several hours it assumed a posture as though it was going to vomit. This description fits in with the picture observed in cattle following excessive doses of hexachlorophene.

Untoward effects have never been reported after the other treatments carried out in the field.

7. Hexachlorophene-piperazine complex in sheep

The one oral dose given (15 mg/kg: Table 32) was not followed by symptoms of intoxication.

The complex was administered four times by subcutaneous injection as a suspension of 1 gram per 3 ml olive oil, which in two instances contained 2% aluminum monostearate (Tables 32 and 33). Signs of a systemic toxic action were not observed. The injection of the hexachlorophene-piperazine complex at a dose rate of 30 mg/kg in sheep 45687 did not result in a swelling at the site of injection, but severe tissue reactions occurred with higher dosages. The reactions in the sheep 45695 and 45688, which received 40 and 50 mg/kg respectively, were similar to those observed after injections of G-11 dissolved in Tween 80. The thickening of the whole leg, which developed after the injection at the thigh, subsided only slowly in the course of a few weeks. The injection of about 35 mg/kg in sheep 1546 resulted not only in considerable swelling of the upper leg, but also in a massive necrosis near the site of injection.

8. G-11S

Three cows received G-11S by the oral route at dose rates of 5, 10, and 15 mg/kg respectively (Tables 17a and 17b). The treatments were tolerated without any sign of intoxication. The milk yield of these animals before and after treatment is presented in Table 39.

A drop in milk yield may be noticed in cow 1744 on the first two days, and a slight drop in cow 1738, on the first day, after treatment. Since the mean milk production of the cows was practically unaffected by treatment with G-11S, it seems doubtful whether the drop of short duration following treatment in two of the animals is of any significance, especially since the drop was most marked in the animal which had received the lowest dose.

Limited trials with guinea pigs showed that G-11S is of about the same order of toxicity as G-11. The drugs were given by subcutaneous injection as solutions in olive oil (100 mg/ml), which had been sterilized at 150°C for one hour. G-11 at a dose rate of 100 mg/kg resulted in death of two out of four guinea pigs, while G-11S at the same dose rate killed one of two animals. At the dose rate of 200 mg/kg, G-11 killed both guinea pigs which received the injection, and the same results were obtained with G-11S.

9. "HETOL" IN CATTLE

The cows 17397 and 364024 (Table 21), which received 225 mg/kg of 1,4-di(trichloromethyl)benzene, showed a slightly diminished appetite the day following treatment: they consumed their hay ration somewhat slower than normally, but cattle cakes were readily eaten. These were the only animals which were treated with "Hetol" after the morning feeding. Cow 712068 refused to eat cattle cakes on the morning of the first day after treatment with 225 mg/kg of 1,4-di(trichloromethyl)benzene, and this animal consumed only a small quantity of hay. The appetite was again normal in the afternoon. Slight diarrhoea was observed in this cow the second day after treatment.

The other treatments with "Hetol" have been perfectly tolerated.

10. "HETOL" IN SHEEP

Although food was not withheld either before or after medication, no serious symptoms have been observed after administration of 160 mg/kg of 1,4-di(trichloromethyl) benzene. The sheep 40817, 40832, and 40834 (Table 34), which were housed together in one pen, showed diminished appetite on the 2nd, 3rd, and 4th day after medication, and their faeces were dryer than normally. Abnormalities were not observed in the other three sheep. However, these animals were housed together with untreated sheep, and consequently diminished appetite of individual animals may easily have been overlooked.

X DISCUSSION

Examination of the tables shows that the mean egg count in the week of treatment is often considerably lower, and sometimes higher than the preceding mean counts. The daily egg counts, which have been omitted in the tables, show that the number of eggs often begins to fall two to three days after medication. In order to avoid symptoms of intoxication being overlooked at the week-end, treatment has often been carried out in the beginning of the week. In those cases the last daily egg counts in the week of treatment often were very low when highly active drugs were involved. This drop in egg count in the course of the week has often considerably affected the mean count. In some cases a very marked increase in the number of eggs passed in the faeces was observed in the first days following treatment, especially in sheep. These peaks in daily egg counts were responsible for the relatively high mean counts which have sometimes been recorded in the week of treatment.

Often an inconstant value of the mean faecal egg count can be observed in the first weeks after purchase of the experimental animals. This period of adaptation, which has to be taken into account when the course of the mean weekly egg count is used as a guide in assessing the efficacy of treatment, probably was mainly a consequence of the transport of the animals to the market where they were bought.

As naturally infected animals have been used in these experimental studies, it has never been possible to know how many parasites were present in their livers before treatment. It will be seen from Table 1 that generally a relationship seems to exist between the number of eggs per gram of faeces and the number of mature flukes in the liver. The egg counts before treatment therefore appear to give a rough idea as to the extent of the worm burden before medication. However, this correlation was considered to be insufficiently reliable to base thereon an assessment of the percentage of mature flukes which have been killed by treatment. Another reason for not using the data of Table 1 for estimating the number of mature flukes before medication, is the fact that often several weeks have elapsed between faecal examination and slaughter in the animals mentioned in this table. Nevertheless, by means of pre- and post-treatment egg counts, supported by *post-mortem* worm counts if possible, the approximate evaluation of fasciolicidal efficacy does not present insuperable difficulties, especially when different drugs are compared.

The number of flukes in experimentally infected animals before medication is also unknown. MONTGOMERIE (1928*a*) and others have shown that the number of worms after administration of a known number of encysted metacercariae varies widely, so that only the theoretical maximum number of flukes is known. Experimental infections are indispensable for an exact evaluation of the efficacy of drugs against

young flukes at different ages. As the search for drugs, which are highly efficient in killing mature flukes was the main object of these investigations, chronically infected animals were bought, which present the important advantage that the drugs were tested against the flukes in animals which are comparable with the majority of those which have to be treated in the field.

Chicken tapeworms are remarkably resistant towards the remedies which are successfully employed against most cestodes in mammals. Only a few drugs have been found to be highly effective against *Raillietina* and *Davainea*; to these drugs belong hexachlorophene and dibutyl tin salts. Thus when hexachlorophene was also found to be an outstanding fasciolicide, it seemed worth while to test di-n-butyl tin dilaurate for its activity against *F. hepatica*. The latter drug seems indeed to have shown some activity against mature liver flukes in cattle, but it is clear that a satisfactory effect could only be expected from the use of much higher doses. An increase of the dose rate, however, seems to be hazardous. BARNES & MAGEE (1958) have reported that salts of dibutyl tin are toxic to rats, mice and rabbits, but not to guinea pigs. A single oral dose of 20 mg/kg of dibutyl tin dichloride was found by BARNES & STONER (1958) to produce a visible bile duct lesion in the rat, while a dose of 50 mg/kg made a rat ill, and a single dose of 200 mg/kg killed two out of eight rats. The acute effects of the dilaurate have been compared by these authors with the dichloride, and no significant differences in response have been observed. Although we have not observed signs of intoxication in the experimental cattle after administration of di-n-butyl tin dilaurate, the reported observations on small laboratory animals show that it might be dangerous to administer the drug at much higher than 30 mg/kg. Further experiments with di-n-butyl tin dilaurate have therefore not been carried out.

In contrast with dibutyl tin salts, dioctyl tin salts have been found by BARNES & STONER (1958) to be non-toxic if given by mouth to mice, guinea pigs and rabbits in doses up to 400 mg/kg for three to four consecutive days. As HARANT *et al.* (1957) have reported that both di-n-octyl tin dilaurate and di-n-octyl tin dichloride at high doses can successfully be employed against the tapeworm *Hymenolepis fraterna* in mice and rats, these compounds have also been tried against *Fasciola hepatica*. The results have not been encouraging, and since the organic tin compounds are expensive, it was considered that higher doses would be uneconomic in practice. Therefore no further experiments with dioctyl tin compounds have been carried out.

Treatment of cow 360406 with bithionol at a dose rate of about 39 mg/kg was unsuccessful. UENO *et al.* (1960), however, have reported that complete eradication of adult liver flukes in cows had been achieved in Japan by oral administration of 35–55 mg/kg of bithionol. WATANABE's remark (1958) that most of the liver flukes in Japan are *Fasciola* species other than *F. hepatica*, possibly accounts for the discrepancy, although differences in breed of cattle and in age of the experimental

animals also may have been important factors. In these studies, when treatment with a dose of about 39 mg/kg appeared to be a failure, further tests with bithionol were not performed because treatment of cattle with much higher doses was considered to be too expensive in practice.

Since the results of MONTGOMERIE's investigations were published, an oral dose of 1 ml carbon tetrachloride for sheep has been recommended in several books on veterinary parasitology, and this dose has probably been applied on a large scale for many years. The results of the treatments of sheep 479, however, show that the efficacy of this treatment is not always satisfactory. It might be possible that the mode of application influences the efficacy, and that the presence of liquid paraffin (synonyms: Paraffinum Liquidum, liquid petrolatum) has retarded absorption of CCl_4, but OLSEN's results (1948) also were not always satisfactory, although the dose of 1 ml CCl_4 was administered in the same way as had been done by MONTGOMERIE (1926).

The experience that oral doses of up to 5 ml carbon tetrachloride did not yield satisfactory results against mature *F. hepatica* in cattle agrees with published reports (WILSON & BLAKEMORE, 1930; GRAWERT & EICHMANN, 1930), but the results obtained by intramuscular injection of CCl_4 in cattle are very different from those mentioned by nearly all other authors. Only WINTERHALTER (1961) reported that intramuscular injection of a mixture of CCl_4 and liquid paraffin was ineffective against liver flukes in cattle, if no hyaluronidase was added to the mixture. It is difficult to understand why several authors have found this treatment to be very effective, whilst only WINTERHALTER's results agree with ours. As most authors have determined efficacy by *post-mortem* examination, there can hardly be any doubt that the treatment has been effective. It might be possible that the sensitiveness of liver flukes in cattle to CCl_4 varies in different parts of the world. It is not always clear from the available sources that the experimental animals were infected with *Fasciola hepatica*, and not with one or more other *Fasciola* species. In connection with this matter it is interesting that not so long ago some doubt has arisen in the Gulf States of the U.S.A. (Sinitsin, after SHAW, 1946), and in Japan (WATANABE, 1958), whether the common liver flukes in these parts of the world should be classified as *Fasciola hepatica*, as has apparently been done for many years. The possibility that *Fasciola* species other than *F. hepatica* were involved in the investigations of some authors on the therapeutic value of carbon tetrachloride and other drugs apparently cannot be ruled out. It also does not seem to be impossible that different strains of *F. hepatica*, which cannot be distinguished on morphological grounds but which respond differently to drugs, are present within the wide-spread area of distribution of the species (Western and Eastern Europe, Asia, Australia, America).

Another factor, which may be of importance, is the duration of infection, which is, in areas where fascioliasis is endemic, closely related to the age of the host. It seems possible that efficacy has mainly been determined in young animals, in which the

flukes are probably relatively readily killed. Data on the age of the experimental animals could often not be ascertained by the writer as the available information was insufficient. The body weights of the cattle used by PEARSON & BORAY suggest that a high percentage of the animals was young. Their results, however, are not comparable with ours because the mode of application of CCl_4 was different.

The possibility has also been considered that the degree of purity of CCl_4 influences the results, but a sample of commercial grade did not yield better results than analytical reagent grade.

As the mixture used by KOVÁCS (1959, 1959*a*) also contained lidocaine, this constituent has been tested separately in the form of its water soluble hydrochloric acid salt. The treatments did not result in an obvious decrease in mean egg count.

Whatever the cause of the mentioned discrepancies may be, it can be concluded that intramuscular injection of a mixture of equal volumes of carbon tetrachloride and liquid paraffin in doses up to 60 ml is insufficiently effective against mature flukes in Dutch cows with chronic liver fluke infection, and that this treatment should therefore not be recommended in this country.

As "Vitan" is a proprietary preparation of secret composition, it is uncertain whether the used preparation is identical with that employed under the direction of MAROTEL. The present results indicate that "Vitan" is of little or no value for the treatment of cattle under the circumstances which prevail in the Netherlands.

The dose of "Freon 112", which was administered to cow 326539 (Table 18), was about 330 mg/kg or about 0.15 g/lb. When the drug was melted at 25°C, it appeared that 330 mg would correspond to about 0.2 ml, and it thus appears that the dose administered to this cow could not have been very much lower than the dose which was found by DEMIDOV (1959*a*) to be effective against liver flukes in cattle. Moreover, the dose rate was equal to what had appeared to be the therapeutic dose rate in sheep. Nevertheless the reduction in number of eggs passed in the faeces of the mentioned cow after medication was unsatisfactory. A high efficacy against *F. hepatica* in chronically infected cows could possibly be achieved with doses higher than 200 g of Freon 112 per animal, but the treatment would be rather expensive, and the bulk required to treat a great number of animals would be a nuisance.

It seems yet to be unknown whether Freon 112 passes into the milk of treated animals. This matter is of importance because the drug has a strong smell.

The reduction in egg count observed after treatment of cattle with a dose of 10 mg/kg of hexachlorophene is much more pronounced than the reduction obtained after the treatments with the above mentioned drugs. It seems likely that this dose of hexachlorophene would be sufficient to cure cattle of chronic fascioliasis.

The results of *post-mortem* examination show that 8 out of 16 cows treated with

15 mg/kg of G-11 seemed to be freed of mature flukes; in the livers of the remaining eight cows one fluke was found in each of six animals, two flukes in one animal, and seven flukes in one animal. It may be concluded that by administration of 15 mg/kg of hexachlorophene to cattle generally all or nearly all mature flukes are killed. The accompanying reduction in number of eggs passed in the faeces, virtually to zero, is of great importance. It means that infection of the intermediate snail host, resulting from liver fluke eggs in cattle faeces, could practically fully be prevented by treatments of cattle with hexachlorophene, whereas it is clear that no satisfactory results could be expected of preventive treatment of cattle with the conventional drugs.

The results of treatment of seven cows with 20 mg/kg of G-11 suggest that all mature flukes have been killed in these animals. Although this dose generally has been perfectly tolerated by our experimental animals, the experiments in which higher doses were involved suggest that application of 20 mg/kg in the field is not advisable. Overestimation of the body weight of cows then would result in a dose which is close to the toxic level, and administration of the double dose by mistake possibly could be fatal.

Subcutaneous administration of hexachlorophene in cows resulted in severe tissue reactions, as has also been observed by OSINGA (1960) and STREHL (1960). It might be possible that less severe tissue reactions would occur if the dose were divided and administered by injections at different sites. We did not try that because it was found that G-11 is at least as effective when it is administered orally, and therefore treatment by injection was abandoned.

Our experience that hexachlorophene is highly effective against mature flukes in cattle, if administered at the dose rate of 10 to 20 mg/kg, was confirmed by FEDERMANN (1959) and NEMESÉRI (1960). OSINGA (1960), however, considered a dose of 10–12.5 mg/kg of G-11 as unsatisfactory against fascioliasis in cattle when the efficacy was assessed by evaluation of clinical response and by faecal examination about four weeks after treatment. The animals were said to have been suffering from clinical fascioliasis before treatment, but as the symptoms of fascioliasis in cattle are not characteristic, and as no pre-treatment egg counts have been given, the statement that the liver flukes were responsible for the poor condition of these animals is not convincing. Post-treatment egg counts have not been recorded either, and no information is therefore available on the extent of the residual infection. The only fact to go by is the presence of liver fluke eggs in the faeces of 67% of the animals about four weeks after oral administration of 10–12.5 mg/kg of G-11, and in the faeces of eight out of nine cattle which had received 10–15 mg/kg of G-11 by subcutaneous injection. Since the body weights of the animals were estimated, a number of animals has probably received a dose of about 10 mg/kg. If it is considered that in our own experiments probably about half the number of animals treated by administration of 15 mg/kg of G-11 was completely cleared of mature flukes, it can be concluded that the percentage of animals found positive by OSINGA for flukes after oral administration of

G-11 does not seem to conflict with our findings. In our experiments no more than a few flukes survived after a dose of 15 mg/kg of G-11, and therefore it does seem peculiar that the cattle in OSINGA's experiment showed no satisfactory clinical improvement after treatment with a somewhat lower dose, unless the livers were so badly affected by chronic infestation that the animals were not able to recover, even if they got rid of their flukes. In the latter case clinical improvement is useless as a criterion in assessing the effectiveness of treatment against liver flukes.

We have observed either no side effects, or only negligible effects, after about 1,200 treatments of cattle with estimated doses of 12.5 or 15 mg/kg of G-11. The drug was given by mouth as an aqueous suspension. Symptoms of intoxication were not observed in our laboratory experiments with doses up to 20 mg/kg of G-11, with the exception of three of the eight cows which were treated shortly before or after parturition. Similar results have been reported by FEDERMANN (1959) and NEMESÉRI (1960) after administration of G-11 to cattle by the oral route, and by STREHL (1960) after subcutaneous injection. The symptoms of intoxication which we have observed after administration of 30 mg/kg of G-11 and higher doses, are rather similar to those reported by KERR (1948) and by GUILHON & GRABER (1961*a*) in chickens, and by GUILHON & GRABER (1961) in sheep, although we have not noticed all symptoms described by GUILHON & GRABER.

OSINGA (1960) reported to have observed indigestion with diarrhoea and decreased milk yield after administration of hexachlorophene to cattle by the oral route at the dose rate of 10 to 12.5 mg/kg, while one animal showed severe grass tetany-like symptoms. In our experiments a dose of 15 mg/kg of G-11 did not materially affect milk production in cows, and this dose was higher than that applied by OSINGA. The description of symptoms as "grass tetany-like" is rather vague. We have observed some of the symptoms of grass tetany as described by HUTYRA *et al.* (1949), but several of the symptoms which are characteristic for this disease, as for instance nervousness, fibrillary twitching of muscles, convulsive attacks, and excitement, have never been exhibited by our experimental animals. We have administered a solution of hexachlorophene in propylene glycol only to one calf and two yearling cattle, and no signs of intoxication were observed at 20 mg/kg of G-11. As this number of animals is very small, it still might be possible that administration of G-11 together with organic solvents, as has been practised by OSINGA, may increase the acute toxicity of hexachlorophene, for instance by enhanced absorption of this drug from the intestine.

It would seem that the excitation observed by NEMESÉRI (1960) for two to three hours after parenteral administration of 15 mg/kg of hexachlorophene in alcohol or liquid paraffin probably has been due to pain at the site of injection.

It is remarkable that GUILHON & GRABER (1961) observed intoxications and even death following treatment of cattle with 20 to 25 mg/kg of hexachlorophene. The mode of administration and the state of health of these animals before treatment were not mentioned. For the treatment of animals which are suffering from severe clinical

fascioliasis it seems advisable to give an initial dose of not much more than 5 mg/kg. If the animals have somewhat recovered a few weeks later, treatment may be repeated with a higher dose of G-11.

In contrast with the results obtained in cattle, the effectiveness of hexachlorophene against *F. hepatica* in sheep appears sometimes to be more or less unsatisfactory. It is apparent that G-11 is generally at least as effective in sheep as it is in cattle, but the results are less uniform in the former, and the flukes appeared to resist treatment in certain individual sheep. When faecal examination indicated that treatment was partially or completely ineffective another mode of application was tried in these individuals.

Consistent results were only obtained after treatment of sheep with G-11 solubilized in water with Tween 80. LIENERT (1960*c*) has shown that liver flukes implanted subcutaneously in the loin of rats are killed by oral administration of G-11 to the experimental host, apparently by sucking blood which contained the drug. It thus seems likely that liver flukes in the bile ducts of medicated cattle and sheep also are killed by sucking hexachlorophene containing blood, although the possibility cannot be ruled out that the drug also acts by mediation of the bile. In order to kill the flukes, a sufficiently high concentration of hexachlorophene has to be reached in the blood, and possibly in the bile. This concentration depends on the rate of absorption of the drug, and on the rate of excretion, and probably also of conversion, on the other hand. It was therefore considered that the efficacy of administration of G-11 by the oral route could be improved if the absorption of the drug from the intestine could be enhanced. The mode of absorption of hexachlorophene presents a problem, for the drug is practically insoluble in the acid and neutral contents of the abomasum and the intestine respectively. The writer found, however, that hexachlorophene could be dissolved in a sample of bile from the gall-bladders of cows up to a concentration of about 1% at 39°C. Probably the drug was dissolved by solubilization. The writer believes that bile plays an important role in the absorption of hexachlorophene by its solubilizing action. As GREGG & ZOPF (1951) have found that hexachlorophene is readily solubilized in water with Tween 80, treatment with G-11, solubilized in this way, was tried, because it was hoped that Tween 80 might complete the action of bile by enhancing the absorption of hexachlorophene. The apparent success of this mode of application seems to indicate that solubilized hexachlorophene is more readily absorbed than the drug in the other employed forms. As a consequence of the assumption that bile acts as a solubilizer for hexachlorophene and in this way promotes absorption of the fasciolicide, the thought suggests itself that sheep, which were not cleared of their flukes by treatment, might not have secreted a sufficient amount of bile constituents at the time when hexachlorophene was present in the intestine.

As oral administration of hexachlorophene, suspended in water, at the dose rate of 5 or 10 mg/kg yielded very inconsistent results in sheep, this mode of application was

not tried with higher doses, because it was considered that consistent results could not be expected unless perhaps the toxic level was approached. For the same reason oral doses higher than 15 mg/kg of G-11, dissolved in propylene glycol, have not been tried. The dose of G-11, administered by mouth as the oily solution, has not been given in excess of 15 mg/kg, because the increased amount of vegetable oil would probably cause diarrhoea and a more rapid passage of the active drug through the intestine, resulting in decreased absorption. Because of the tissue reactions doses higher than 15 mg/kg of G-11 have not been administered by subcutaneous injection, since it is clear that much higher doses would be required to obtain consistent results.

For the treatment of sheep with hexachlorophene oral administration of the solubilized drug seems to be the method of choice. As has already been found by GREGG & ZOPF (1951), it was found that the fluid preparation deteriorates within a few weeks by mould growth. It appeared that deterioration could not be prevented by the addition of "Nipagin", which is in accordance with reported findings, which have been reviewed by WHITTET (1959), that the parahydroxybenzoate derivatives are incompatible with nonionic emulsifiers, and that they react with sorbitan derivatives such as Tween 80. We found that the solubilized hexachlorophene preparation can effectively be preserved by the addition of orthooxyquinoline sulphate ("Superol") at the concentration of 5 mg/ml. There are no indications that this preservative interferes with the action of hexachlorophene on the liver flukes, or that it increases the toxicity of the preparation.

A dose of 30 mg/kg of hexachlorophene, administered orally in the solubilized form, seemed also to kill immature flukes in sheep. It remains to be determined, by means of treatment of experimentally infected sheep, what is the minimum age at which the flukes can be killed. The lowest dose at which the preparation is nearly completely effective against mature and immature flukes also has not yet been determined.

The results obtained by most authors by medication of sheep with hexachlorophene are not comparable with those of the present writer. FEDERMANN (1959), who employed a watery suspension for oral administration, has obtained excellent results with doses of 15 and 20 mg/kg of G-11. This mode of administration has not been applied in our experiments at dose rates higher than 10 mg/kg. For similar reasons the results obtained by GUILHON & GRABER (1961) cannot be compared with ours. STREHL (1960) and NEMESÉRI (1960) have employed hexachlorophene in media which are different from those used in our experiments. According to WATANABE (1958) the liver fluke in the experiments of UENO *et al.* (1960*a*) was possibly not *Fasciola hepatica*.

The writer did not perform experiments on the toxicity of hexachlorophene in sheep. It appeared that on the whole sheep tolerate high dose rates as compared with cattle. Generally doses up to 30 mg/kg of G-11 have been very well tolerated by sheep. The cases with fatal outcome occurred after administration of 15 or 30 mg/kg in animals with extremely high burdens of mature flukes. This suggests that either the

liver was in such a bad state that detoxication of the chlorinated phenol was considerably hampered, or that the great number of killed flukes has caused a fatal intoxication. It seems also possible that both factors together were responsible for serious disease and death. In the light of these experiences it seems advisable in practice to administer to heavily infested sheep an initial dose no higher than 5 mg/kg of G-11. In many animals this dose will be sufficient to kill all or nearly all mature flukes. A few weeks later treatment may be repeated with a higher dose in those animals in which treatment with 5 mg/kg has yielded unsatisfactory results.

For above mentioned reasons, data reported by FEDERMANN (1959), STREHL (1960), and NEMESÉRI (1960) on the tolerance of sheep towards hexachlorophene cannot be compared with ours. OSINGA (1960) stated that he had treated sheep suffering from clinical fascioliasis with doses of 10 to 12.5 mg/kg of G-11. It seems probable that the intolerance, which was observed in several of the animals, was due to the parasitic disease, and that in these cases the initial dose has been too high. We have found no indications that the use of propylene glycol as a vehicle considerably increased the toxicity of hexachlorophene for sheep. No information is available to the writer as to the state of health and the degree of infestation of the sheep treated by UENO *et al.* (1960*a*), and the reason why the animals showed side effects after a dose of 20 mg/kg of G-11 can therefore be no subject of discussion. GUILHON & GRABER (1961) reported that three out of four African sheep, which were not infected with liver flukes, died after an oral dose of 40 mg/kg of G-11. One of the animals that died was a lamb in good condition, and the two others were sheep emaciated by the presence of a heavy burden of gastro-intestinal parasites. The tolerance observed by GUILHON & GRABER in French sheep does not seem to be very different from that which we have experienced.

The tissue reactions following subcutaneous administration of a solution of G-11 in olive oil generally were considerably less severe in sheep than they were in cattle. The same difference has been observed by STREHL (1960), although vehicles have been used which were different from ours. Apparently the tissue injury was caused by hexachlorophene, while the nature of the vehicle had little or no influence. FEDERMANN (1959) observed slight to considerable swelling at the site of injection in sheep. In our experiments a severe tissue reaction was observed in only one out of nine sheep, and STREHL observed a local reaction in one out of seven sheep which had received G-11 by subcutaneous injection. It may be concluded that the frequency of local reactions in sheep following subcutaneous injection of hexachlorophene is rather high, so that one should advise against this mode of administration in the field.

The hexachlorophene-piperazine complex was tested for its efficacy against liver flukes in sheep at the stage of the experiments when a mode of administration of hexachlorophene, which yielded consistent results, had not yet been found. It was

considered that better results might possibly be achieved if hexachlorophene could be applied in the form of a depot under the skin, which would release the active compound for a long period of time, and cause chronic poisoning of the flukes, eventually leading to death of the parasites. The results did not suggest that the complex compound had advantages over G-11 at the modes of administration which we have practised. As the tissue reactions after subcutaneous injection of 35 mg/kg or more of the complex were severe, experiments with this drug were discontinued when it was found that oral administration of solubilized hexachlorophene yielded very encouraging results.

GOULD *et al.* (1953), who studied the effect of G-11 and certain related bis-phenols on enzyme systems of bacteria, observed a synergistic effect when a small amount of an inactive bis-phenol was added to G-11. Since the therapeutic index of hexachlorophene in the treatment of cattle against liver flukes is not high, attempts were made to increase its therapeutic index by the addition of other phenols. The results seem to indicate that addition of either 50 mg/kg of sodium pentachlorophenate or 7 mg/kg of dichlorophene to a dose of 5 mg/kg of hexachlorophene has somewhat enhanced the effectiveness of hexachlorophene against *F. hepatica* in cattle, although the effect is not very convincing, especially since the results of comparable treatments with G-11 alone cannot yet be presented. Further work on this subject is in progress, and some preliminary results seem to indicate that combination of 10 mg/kg of G-11 with either 10 mg/kg of G-4 or 50 mg/kg of sodium pentachlorophenate is very effective against mature *F. hepatica* in cattle.

KERR & GREEN (1953) tested seven compounds of the diphenyl methane series, one diphenyl propane, and one diphenyl ether against poultry tapeworms. They found two compounds to be highly effective: hexachlorophene and bis-(2-hydroxy-3,4,5,6-tetrachlorophenyl)-oxide. The results suggested that the nature of the bridge, to which the two phenyl groups are attached, is not of great importance with respect to anthelmintic activity, and that probably there is little difference between a methane and an ether linkage.

This surmise prompted us to test compound G-11S when we had established the high activity of G-11 against liver flukes. The activity of G-11S against *F. hepatica* in cattle seemed to be similar to that of G-11. As the toxicity of both drugs also seemed to be of about the same order when tested on a small scale in guinea pigs, there seemed little to choose between the two. Hexachlorophene is readily available, and therefore this drug has been used in all further experiments.

The high fasciolicidal activity of G-11S suggests that testing halogenated bis-phenols, which are closely related to G-11, might be fruitful.

1,4-Di(trichloromethyl)benzene has shown very good activity against mature

F. hepatica in cattle at the dose rate of 225 mg/kg. The high efficacy of a dose of 136 mg/kg, as reported by LÄMMLER (1960), was not confirmed in our experiments, and satisfactory results have also not been obtained by administration of 150 mg/kg. We have carried out only one treatment with 80 g "Hetol", the maximum dose advised by LÄMMLER, and all other doses were considerably higher.

It may be concluded that doses of about 225 mg/kg of 1,4-di(trichloromethyl) benzene are required to obtain satisfactory results in the treatment of chronically infected cows in the Netherlands. This means that often more than 150 g "Hetol" would have to be administered per cow. The treatment would then probably be rather expensive. Moreover, such high doses should not be advised without further experience on the tolerance of cattle towards the drug.

The body weights of the cattle treated by LÄMMLER are low as compared with those of our experimental cows of the Friesian breed. The highest body weight which LÄMMLER has recorded is 510 kg; the weights of the other experimental cattle were nearly all considerably lower. It seems therefore likely that LÄMMLER has determined the efficiency of treatments with "Hetol" mainly in young cattle, in which the flukes are probably relatively easy to kill. Another fact, which might account for the discrepancy as regards the therapeutic dose as determined by LÄMMLER and by us, is our finding that egg production by surviving flukes may be suppressed for a period of up to five weeks following medication of cattle with "Hetol". As this phenomenon was observed in cows which had been housed continuously for more than a year, it seems very unlikely that the rise in egg count following the initial drop after treatment was due to the presence of immature flukes which had grown mature after medication. Since LÄMMLER performed the last faecal examination four weeks after treatment, it thus may be possible that this author has somewhat overestimated the efficacy of treatment. A third point to be considered is the very low value of the egg counts recorded by LÄMMLER. This suggests either that the worm burdens were very low, or that the employed egg counting technique did not meet the requirements. If the former possibility is the true one, it could mean that the animals had been exposed to infection only for a short period. In the latter case one wonders whether a constant percentage of eggs was missed, a matter of great importance when a drop in egg count is assessed. Unfortunately LÄMMLER did not mention the time of the day when the faecal samples were collected.

Our results with six sheep suggest that a dose of 160 mg/kg of 1,4-di(trichloromethyl)benzene is sufficient to kill all or nearly all mature flukes in this host. The results which were obtained with "Hetol" in sheep by LÄMMLER (1960) thus have been confirmed in our experiments.

Since multiplication of the liver fluke occurs in the intermediate snail host, it is clear that prophylaxis of fascioliasis by means of chemotherapy can only be achieved if drugs are used which very effectively reduce the number of fluke eggs passed in the

faeces. A drastic reduction in contamination of pastures with fluke eggs will considerably diminish the opportunity for the snails to become infected.

Our experiments have indicated that three drugs seem to be sufficiently active against mature flukes in cattle to fulfil the requirement: G-11, G-11S, and "Hetol". The same applies to G-11 in the solubilized form and to "Hetol" against mature flukes in sheep .All the other treatments can be considered to be insufficiently effective to be of value in the prophylaxis of fascioliasis.

Field experiments are in progress to test the prophylactic value of three treatments a year with hexachlorophene. The results so far obtained are encouraging.

These three drugs also seem to provide a considerable improvement in the curative treatment of fascioliasis in cattle. Hexachlorophene in the solubilized form seems to be an important advance for the alleviation of both acute and chronic fascioliasis in sheep. "Hetol" also seems to be very valuable for the treatment of chronic fascioliasis in sheep.

At the present time, however, application of G-11, G-11S, and "Hetol" on a large scale cannot be advocated. The tolerance of cattle towards the required dose of "Hetol" is still insufficiently known; the fate of 1,4-di(trichloromethyl)benzene and of G-11S in the body of treated animals seems to be unknown, and that of G-11 is still a subject of study. The results so far obtained with the latter drug are promising: indications have been found that hexachlorophene is rapidly excreted and converted, that it does not accumulate in the body, and that it does not pass into the milk. If these results are confirmed in further experiments, hexachlorophene could be recommended as the drug of choice under the circumstances which prevail in the Netherlands. The drug should be administered only orally, as a watery suspension, to cattle, and preferably in the solubilized form, to sheep. According to our experience the costs of the required amount of the drug do not need to be higher than about *f* 0.10 per 100 kg body weight.

The margin of safety of hexachlorophene is not very wide, and therefore the drug has to be accurately dosed. Since a suspension of the drug in water settles rapidly, while the sediment is not easily resuspended, one should advise with great emphasis against the preparation of a drench for a number of cattle, even if it is stirred or shaken frequently. We have found that the risk of faulty dosing can easily be avoided by the use of hexachlorophene tablets which rapidly disintegrate in water, and by preparing a drench for each animal separately. Another method, which might appear to be convenient for administering an acurate dose to each individual animal, is spreading a measured amount of a hexachlorophene paste on the tongue.

SUMMARY

A detailed and critical survey of the literature on the existing treatments against *Fasciola hepatica* in cattle and sheep, with special reference to the methods which have been employed to determine the efficacy of drugs against the mature parasites, has been given. It appeared that several drugs and different modes of application have been advocated, but that nearly every treatment has been found by one or more investigators to be of little value in the control of mature *F. hepatica*. The opinions on the safety of the different treatments also vary widely.

As reports on the efficacy and toxicity of the available fasciolicides are so conflicting, it was considered necessary to revaluate some of the described treatments, and also to endeavour to develop new and better treatments.

Since medication of cattle against liver flukes seemed to present more difficulties than that of sheep, and also because cattle are more important from the economical point of view than sheep in the Netherlands, the screening of preparations has mainly been carried out with infected cattle. At a later stage new treatments have also been tried in sheep. The liver lesions, which result from chronic infection, have been pointed out by several authors as being responsible for unsatisfactory results of medication. As it was not only the purpose to find treatments which efficiently control liver flukes in comparatively recent infections in calves and lambs, but also those which are highly effective against mature flukes in adult animals with chronic infections, the screening of preparations has been performed with chronically infected cows and not with experimentally infected animals. Pre- and post-treatment faecal egg counts, and *post-mortem* examinations have been carried out to evaluate the results of treatments.

After some described techniques for the determination of the number of *Fasciola* eggs per gram of faeces had been found unsatisfactory, a new technique was designed, which has been described earlier. By means of the new egg counting method the number of eggs per gram of faeces has been determined in samples which have been obtained from the rectum of cattle with intervals of one hour. Without exception a diurnal fluctuation of the number of eggs was found: a marked increase in the morning followed by a decrease in the afternoon. Probably this pattern is repeated every day in a similar way in all infected cattle. The fluctuation has some consequences of considerable practical importance: when egg counts are used for assessing the efficacy of drugs against mature flukes in cattle, it is necessary always to collect faecal samples from the rectum at a fixed hour, preferably in the beginning of the afternoon. For this reason fresh faecal samples have always been collected from cattle between 1.30 and 2.00 p.m. A diurnal fluctuation of the number of *Fasciola* eggs in the faeces was not found in sheep.

Even when faecal samples were always collected at a fixed time, the variation in the number of eggs per gram of faeces from day to day generally was considerable. When, however, the mean value was determined from five daily egg counts within a week, it appeared that this mean count generally did not show very great variation in untreated animals. By determining the weekly mean count during periods covering several weeks before and after treatment, very valuable information on the activity of drugs against mature flukes could be obtained. The advantages of this method, as compared with the conventional procedure of a few examinations of faecal samples, which have been obtained at any given time, have been outlined.

Results obtained in cattle

Twenty-four preparations, including bithionol, were found ineffective at the employed dosages. More or less effective against mature liver flukes in cattle appeared to be: dibutyl- and dioctyl tin compounds, carbon tetrachloride, "Vitan", Freon 112, hexachlorophene (G-11), G-11S, and "Hetol". The efficacy of the alkyl tin compounds, "Vitan", Freon 112, and carbon tetrachloride was unsatisfactory at the employed dosage rates. Intramuscular injection of the latter drug in doses up to 30 ml resulted in no more than only a slight reduction in the number of fluke eggs in the faeces. Hexachlorophene, G-11S, and "Hetol" were found to be highly active against mature flukes in cattle.

Hexachlorophene (G-11)

At a given dose rate comparable results were obtained with either administration per os or with subcutaneous injection. Indications were not found that the nature of the vehicle influences anthelmintic activity.

A dose of 5 mg/kg of G-11 resulted in a slight or moderate reduction in the number of eggs in the faeces, and probably also in the number of mature flukes.

A dose of 10 mg/kg of G-11 very efficiently reduced the number of eggs in the faeces, and apparently a small number of mature flukes remained alive.

A dose of 15 mg/kg of G-11 killed all or nearly all mature flukes. Only in one case the efficacy was not completely satisfactory.

All mature flukes seemed to have been killed by doses of 20 mg/kg of G-11 and higher.

Subcutaneous injection of G-11 dissolved in olive oil gave rise to a considerable swelling at the site of injection. This mode of application has therefore been abandoned after a few trials, and all further treatments have been carried out by administering G-11 by the oral route.

Oral doses of hexachlorophene in aqueous suspension of up to 15 mg/kg have been perfectly tolerated by reasonably healthy cattle of different ages, by cows in advanced gestation, and by cows which had recently calved. Generally an oral dose of 15 mg/kg of G-11 did not seem to affect the milk yield. A dose of 20 mg/kg was nearly always

perfectly tolerated; only within a few weeks before or after parturition cows sometimes appeared to be slightly more sensitive. Symptoms observed in cattle after administration of 20, 30, or 40 mg/kg of G-11 have been described.

Combinations of hexachlorophene and other compounds

Combinations of 5 mg/kg of G-11 and other compounds have not yielded very satisfactory results. Indications have been found, however, that sodium pentachlorophenate and dichlorophene may enhance the fasciolicidal effect of hexachlorophene.

G-11S

The results obtained in three cattle by oral administration of 5, 10, and 15 mg/kg respectively, suggest that the fasciolicidal efficiency of G-11S is about the same as that of G-11. The toxicity of both drugs also seems to be of the same order.

"Hetol"

1,4-Di(trichloromethyl)benzene was found to be highly active against mature flukes in cattle at the dose rate of 225 mg/kg. Symptoms of intoxication of any importance have not been observed at this dosage. Doses of 150 mg/kg of 1,4-di (trichloromethyl)benzene and lower did not yield satisfactory results.

Results obtained in sheep

Two treatments have been carried out in one sheep by oral administration of 1 ml carbon tetrachloride. The result of the first treatment was not completely satisfactory, and the second treatment resulted in no more than a temporary reduction in the number of eggs produced by the flukes. Dioctyl tin dichloride seemed to have only slightly affected the mature flukes in the animal which had received 200 mg/kg of the drug.

Further treatments of sheep have been carried out only with hexachlorophene (G-11), the complex of hexachlorophene and piperazine, and "Hetol".

Hexachlorophene (G-11)

Excellent results have been obtained in sheep by oral administration of 20 or 30 mg/kg of G-11 when the drug was solubilized by means of Tween 80. At 30 mg/kg this treatment seemed also to be very effective against immature flukes in the liver parenchyma. Other modes of administration yielded inconsistent results.

A few sheep with extremely high burdens of mature flukes appeared not to tolerate doses of 15 mg/kg of G-11 and higher, but sheep and lambs which were not suffering from very severe chronic fascioliasis have tolerated doses up to 30 mg/kg of the drug very well.

The local tissue reactions after subcutaneous injection of an oily solution of G-11 generally were less severe than those in cattle. However, tissue reactions appeared to occur rather frequently, and the local injury may sometimes be severe.

Hexachlorophene-piperazine complex

A complex was prepared of the anthelmintics hexachlorophene and piperazine. Application of this complex in sheep did not seem to present advantages over that of hexachlorophene. Local tissue reactions after subcutaneous injection of high doses of the complex in olive oil were severe.

"Hetol"

In limited trials 160 mg/kg of 1,4-di(trichloromethyl)benzene appeared to be practically fully effective against mature flukes in sheep.

It can be concluded that three of the tested preparations are highly effective against mature flukes: hexachlorophene (G-11), G-11S, and "Hetol". By application of these new drugs the curative treatment of chronic fascioliasis could therefore be considerably improved, and it seems that hexachlorophene in the solubilized form could also successfully be employed against young flukes in the liver parenchyma of sheep.

By treatment of infected animals with G-11, G-11S or "Hetol" the passing of *Fasciola* eggs in the faeces can practically be stopped. It should therefore be possible progressively to reduce infection of the intermediate snail host with larval stages of *Fasciola hepatica* by periodic treatment of cattle and sheep with these drugs. It can be expected that in this way liver fluke infection in farm animals can practically fully be prevented.

At the present time, however, application of G-11, G-11S, and "Hetol" on a large scale cannot yet be recommended. The required dose of "Hetol" for sheep seems to be sufficiently safe when the prescribed diet is strictly observed, but little appears to be known about the risk of administering the very high doses which are necessary in cattle. The fate of 1,4-di(trichloromethyl)benzene and of G-11S in the body of treated animals seems to be unknown, and that of G-11 is a subject of study in the Netherlands. Preliminary results, which have been obtained elsewhere on the excretion of hexachlorophene, seem to warrant the expectation that this extremely useful and comparatively cheap fasciolicide can soon be recommended as the drug of choice in the Netherlands.

POSTSCRIPT

Since the completion of this publication very interesting contributions on hexachlorophene have appeared in press. BOSMAN *et al.* (1961) reported favourably on the use of G-11 as a fasciolicide for cattle and sheep in South Africa, while KENDALL & PARFITT (1962) demonstrated the effectiveness of high doses (40 mg/kg) against *F. hepatica* of only 3–4 weeks old in experimentally infected rabbits and sheep.

Studies on the fate of hexachlorophene in rabbits, rats and dairy cows after oral administration of the drug were published by WIT & VAN GENDEREN (1962) of the National Institute of Public Health in Utrecht. In view of their results there no longer seems to be any reason for advising against the use of hexachlorophene (G-11) against liver fluke on a large scale, provided that treatment is not carried out within 5 days before slaughter. A warning, however, should be added. Recent trials in our laboratory have shown that orally administered hexachlorophene in the solubilized form is about twice as toxic to guinea pigs as a suspension of the drug in water. The relatively high toxicity of G-11 in molecular dispersion suggests that it might well be possible that the toxicity of a hexachlorophene suspension depends to some degree on the mean particle size (or the surface area per gram) of the preparation used.

SAMENVATTING

Een uitvoerig en critisch overzicht wordt gegeven van de literatuur over de bestaande behandelingen tegen *Fasciola hepatica* bij rund en schaap, waarbij in het bijzonder aandacht is geschonken aan de methoden die gebruikt zijn om de werkzaamheid van geneesmiddelen tegen de geslachtsrijpe parasieten vast te stellen. Het bleek dat enige middelen en verschillende wijzen van toediening aanbevolen zijn, maar dat bijna iedere behandeling door een of meer onderzoekers van weinig waarde werd bevonden voor de bestrijding van geslachtsrijpe *F. hepatica*. De meningen over de risico's van de verschillende behandelingen lopen ook zeer uiteen.

Daar de mededelingen over de werkzaamheid en de giftigheid van de beschikbare leverbotmiddelen zo met elkaar in tegenspraak zijn, werd het noodzakelijk geoordeeld enkele van de beschreven behandelingen opnieuw op hun waarde te beproeven, en ook te trachten nieuwe en betere behandelingen te vinden.

Aangezien chemotherapeutische behandeling van runderen tegen leverbotten meer moeilijkheden schijnt op te leveren dan die van schapen, en ook omdat runderen in Nederland economisch belangrijker zijn dan schapen, zijn voornamelijk besmette runderen gebruikt voor de selectie van geneesmiddelen. Later werden nieuwe behandelingen ook bij schapen beproefd. De leververanderingen, veroorzaakt door chronische besmetting, zijn door verschillende auteurs verantwoordelijk geacht voor onbevredigende resultaten van behandelingen. Daar het de bedoeling was geneeswijzen te vinden die niet alleen goed werkzaam zijn tegen de leverbotten bij kalveren en lammeren die betrekkelijk korte tijd besmet zijn geweest maar ook tegen geslachtsrijpe leverbotten bij chronisch besmette volwassen dieren, werd voor de selectie van geneesmiddelen gebruik gemaakt van chronisch besmette koeien, en niet van experimenteel besmette dieren. De resultaten van de behandelingen werden beoordeeld door middel van tellingen van de aantallen leverboteieren in de faeces voor en na behandeling en door onderzoek van de proefdieren na het slachten.

Na onbevredigende resultaten te hebben verkregen met enkele methoden welke in de literatuur zijn beschreven voor het bepalen van het aantal eieren per gram faeces, werd een nieuwe techniek ontwikkeld welke reeds eerder is beschreven. Met behulp van de nieuwe methode werd het aantal eieren per gram faeces bepaald bij monsters welke met tussenpozen van een uur werden verkregen uit het rectum van runderen. Zonder uitzondering bleek een dagelijkse fluctuatie voor te komen van het aantal eieren: een aanzienlijke toename tijdens de ochtend werd gevolgd door een afname in de namiddag. Dit verschijnsel herhaalt zich waarschijnlijk iedere dag bij alle besmette runderen. Deze fluctuatie heeft enkele belangrijke praktische consequenties: indien eitellingen worden gebruikt om een indruk te krijgen van de werkzaamheid van ge-

neesmiddelen tegen geslachtsrijpe leverbotten bij runderen dan is het noodzakelijk faecesmonsters steeds op dezelfde tijd uit het rectum te verzamelen, en wel bij voorkeur in het begin van de namiddag. Verse monsters runderfaeces zijn daarom bij dit onderzoek altijd verzameld tussen 13.30 en 14.00 uur. Een dagelijkse fluctuatie van het aantal *Fasciola*-eieren in schapefaeces werd niet vastgesteld.

Zelfs wanneer faecesmonsters steeds op een vaste tijd werden verzameld, bleek het aantal eieren per gram faeces van dag tot dag aanzienlijke variaties te vertonen. Het gemiddelde van vijf dagelijkse eitellingen binnen een week bleek echter in het algemeen van week tot week tamelijk constant te zijn bij onbehandelde dieren. Door deze weekgemiddelden te bepalen gedurende perioden van enige weken voor en na behandeling, werden zeer waardevolle gegevens verkregen over de werkzaamheid van geneesmiddelen tegen geslachtsrijpe leverbotten. Enige voordelen van deze methode ten opzichte van de gebruikelijke werkwijze, waarbij enkele faecesmonsters worden onderzocht welke op willekeurige tijden zijn verzameld, worden besproken.

Resultaten verkregen bij runderen

Vierentwintig verbindingen of preparaten, waaronder bithionol, werden praktisch onwerkzaam bevonden bij de toegepaste doseringen. De volgende middelen bleken min of meer werkzaam te zijn tegen geslachtsrijpe leverbotten bij runderen: dibutyl- en dioctyltinverbindingen, tetrachloorkoolstof, "Vitan", Freon 112, hexachlorofeen (G-11), G-11S en "Hetol". De werkzaamheid van de alkyltinverbindingen, van "Vitan", Freon 112 en tetrachloorkoolstof was onbevredigend bij de toegepaste doseringen. Intramusculaire injectie van tetrachloorkoolstof in doses tot 30 ml had hoogstens een geringe vermindering van het aantal leverboteieren in de faeces tot gevolg. Hexachlorofeen, G-11S en "Hetol" bleken zeer werkzaam te zijn tegen geslachtsrijpe leverbotten bij runderen.

Hexachlorofeen (G-11)

Bij een bepaalde dosering waren de resultaten van toediening per os en van onderhuidse injectie vergelijkbaar. Er werden geen aanwijzingen gevonden dat de vorm waarin G-11 per os werd toegediend (gesuspendeerd, opgelost of gesolubiliseerd) invloed had op de werkzaamheid.

Een dosis van 5 mg G-11 per kg veroorzaakte een geringe of matige vermindering van het aantal eieren in de faeces, en waarschijnlijk ook van het aantal geslachtsrijpe leverbotten.

Een dosis van 10 mg G-11 per kg deed het aantal eieren in de faeces zeer sterk dalen. Blijkbaar bleef een klein aantal geslachtsrijpe leverbotten in leven.

Een dosis van 15 mg G-11 per kg veroorzaakte de dood van alle of van bijna alle geslachtsrijpe leverbotten. Slechts in één geval was de werkzaamheid niet geheel bevredigend.

Alle geslachtsrijpe leverbotten schenen te zijn gedood door doses van 20 mg/kg of hoger.

Subcutane injectie van een oplossing van G-11 in olijfolie veroorzaakte een uitgebreide zwelling op de plaats van injectie. Na enkele experimenten werd deze wijze van toediening daarom verlaten, en alle verdere behandelingen werden uitgevoerd door toediening van G-11 langs orale weg.

Doses tot 15 mg G-11 per kg, toegediend per os in waterige suspensie, werden uitstekend verdragen door redelijk gezonde runderen, door hoogdrachtige koeien, en door koeien die pas hadden gekalfd. In het algemeen leek een orale dosis van 15 mg/kg geen invloed te hebben op de melkgift. Een dosis van 20 mg/kg werd bijna altijd uitstekend verdragen; alleen binnen een paar weken voor of na het kalven bleken koeien soms iets gevoeliger te zijn. Symptomen waargenomen bij runderen na toediening van 20, 30 of 40 mg G-11 per kg werden beschreven.

Combinaties van hexachlorofeen en andere verbindingen

Combinaties van 5 mg G-11 per kg en andere verbindingen hebben niet zeer bevredigende resultaten opgeleverd. Er werden echter aanwijzingen gevonden dat natriumpentachloorfenolaat en dichlorofeen het effect van hexachlorofeen tegen leverbotten kunnen versterken.

G-11S

De resultaten verkregen bij drie runderen door orale toediening van respectievelijk 5, 10 en 15 mg/kg geven de indruk dat de leverbotdodende werkzaamheid van G-11S ongeveer gelijk is aan die van G-11. De toxiciteit van beide verbindingen schijnt eveneens van dezelfde orde van grootte te zijn.

"Hetol"

Een dosis van 225 mg 1,4-di(trichloormethyl)benzeen per kg bleek zeer werkzaam te zijn tegen geslachtsrijpe leverbotten bij runderen. Intoxicatieverschijnselen van enige betekenis werden niet waargenomen bij deze dosering. Doses van 150 mg 1,4-di (trichloormethyl)benzeen en lager leverden geen bevredigende resultaten op.

Resultaten verkregen bij schapen

Een schaap werd twee maal behandeld door orale toediening van 1 ml tetrachloorkoolstof. Het resultaat van de eerste behandeling was niet geheel bevredigend, en de tweede behandeling leverde slechts een tijdelijke vermindering van het aantal eieren in de faeces op. Behandeling van een dier met 200 mg dioctyltindichloride per kg scheen de geslachtsrijpe leverbotten slechts in geringe mate te hebben aangetast.

De overige behandelingen van schapen werden alleen met hexachlorofeen (G-11), het complex van hexachlorofeen en piperazine, en "Hetol" uitgevoerd.

Hexachlorofeen (G-11)

Uitstekende resultaten werden verkregen bij schapen door orale toediening van 20 of 30 mg G-11 per kg indien het middel gesolubiliseerd was met behulp van Tween

80. Bij de dosering van 30 mg/kg leek deze behandeling ook zeer werkzaam te zijn tegen jonge leverbotjes in het leverparenchym. Toediening van hexachlorofeen op andere wijzen leverde niet altijd goede resultaten op.

Enkele schapen die in buitengewoon sterke mate besmet waren met geslachtsrijpe leverbotten bleken doses van 15 mg G-11 per kg en hoger niet te verdragen. Doses tot 30 mg/kg werden echter zeer goed verdragen door schapen en lammeren welke niet aan zeer ernstige chronische distomatose leden.

De locale reacties na subcutane injectie van een oplossing van G-11 in olijfolie waren meestal minder ernstig dan die welke bij runderen optraden. Weefselreacties traden echter vrij vaak op, en waren soms van ernstige aard.

Hexachlorofeen-piperazinecomplex

Een complex werd bereid uit de beide anthelmintica hexachlorofeen en piperazine. Toediening van dit complex aan schapen scheen geen voordelen te bieden ten opzichte van behandeling met hexachlorofeen. Subcutane injectie van hoge doses van het complex in olijfolie veroorzaakte ernstige locale reacties.

"*Hetol*"

In een beperkt aantal proeven bleek 160 mg 1,4-di(trichloormethyl)benzeen per kg praktisch volledig werkzaam te zijn tegen geslachtsrijpe leverbotten bij schapen.

Drie van de onderzochte middelen bleken zeer werkzaam te zijn tegen geslachtsrijpe leverbotten: hexachlorofeen (G-11), G-11S en "Hetol". Door toepassing van deze nieuwe leverbotmiddelen zou de curatieve behandeling van chronische distomatose dus belangrijk kunnen worden verbeterd. Hexachlorofeen in gesolubiliseerde vorm zou bovendien waarschijnlijk ook met succes kunnen worden gebruikt tegen jonge leverbotjes in het leverparenchym bij schapen.

Door behandeling van besmette dieren met G-11, G-11S of "Hetol" kan men de uitscheiding van leverboteieren met de faeces praktisch doen ophouden. Het moet dus mogelijk zijn om besmetting van de tussengastheer (*Lymnaea truncatula*) met larvale stadia van *Fasciola hepatica* in toenemende mate te doen verminderen door periodieke behandeling met deze middelen van alle runderen en schapen op leverbotbedrijven. Het is te verwachten dat besmetting van deze huisdieren met leverbotten op deze wijze vrijwel volledig kan worden voorkomen.

Op het ogenblik moet echter het gebruik van G-11, G-11S en "Hetol" op grote schaal nog worden afgeraden. De behandeling van schapen met "Hetol" in de benodigde dosering schijnt geen nevenwerkingen te veroorzaken indien het voorgeschreven dieet in acht wordt genomen; er blijkt echter weinig bekend te zijn over het risico van behandeling met de zeer hoge doses "Hetol" die nodig zijn bij runderen. Het lot van 1,4-di(trichloormethyl)benzeen en van G-11S in het lichaam van behandelde dieren schijnt niet bekend te zijn, en dat van G-11 wordt nog in Nederland bestudeerd. Enkele voorlopige resultaten, die elders zijn verkregen betreffende de excretie

van hexachlorofeen, lijken de verwachting te rechtvaardigen dat dit buitengewoon nuttige en relatief goedkope leverbotmiddel spoedig kan worden aanbevolen als het aangewezen middel voor toepassing in Nederland.

NASCHRIFT

Na de voltooiing van deze publikatie zijn zeer belangwekkende bijdragen verschenen over hexachlorofeen. BOSMAN *et al.* (1961) berichtten dat G-11 als leverbotmiddel voor runderen en schapen in Zuid-Afrika zeer goed voldeed, terwijl KENDALL & PARFITT (1962) de werkzaamheid aantoonden van hoge doses (40 mg/kg) tegen *F. hepatica* van slechts 3–4 weken oud bij experimenteel besmette konijnen en schapen.

Onderzoekingen over het lot van hexachlorofeen na orale toediening van het geneesmiddel aan konijnen, ratten en melkkoeien, werden gepubliceerd door WIT & VAN GENDEREN (1962) van het Rijks Instituut voor de Volksgezondheid. Gezien hun resultaten lijkt er geen enkele reden meer te zijn het gebruik op grote schaal van hexachlorofeen (G-11) als leverbotmiddel af te raden, mits geen dieren worden behandeld binnen 5 dagen voor het slachten. Een waarschuwing moet echter nog worden toegevoegd. Recente proeven in ons laboratorium hebben aangetoond dat per os toegediende hexachlorofeen in gesolubiliseerde vorm ongeveer twee maal zo giftig is voor caviae als het middel in de vorm van een suspensie in water. Aangezien G-11 in moleculaire dispersie relatief toxisch is, lijkt het zeer goed mogelijk dat de giftigheid van een hexachlorofeen-suspensie enigermate afhankelijk is van de gemiddelde deeltjesgrootte (of het oppervlak per gram) van het gebruikte preparaat.

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TABLE I List of treatments with *alkyl tin compounds*

Compound	Dose	Animal and Treatment
di-n-butyl tin dilaurate	10 mg/kg	cow 360413 (4B)
ditto	20 mg/kg	cow 360406 (4C)
ditto	30 mg/kg	calf 1115 (5A)
di-n-octyl tin dilaurate	400 mg/kg	calf 1115 (5B)
di-n-octyl tin dichloride	200 mg/kg	sheep 45695 (22)

Numbers and capitals within brackets refer to the numbers of tables in which the results of treatments (indicated by capitals) have been given

TABLE II List of treatments with *carbon tetrachloride*

Dose	Mode of application	
	oral	intramuscular
1 ml	sheep 479, 1st treatment (23) sheep 479, 2nd treatment (23)	
3 ml	cow 2273 (6D)	
4 ml	cow 2273 (6A) cow 2273 (6C) cow 327129 (8B)	
5 ml	cow 2273 (6B) cow 325452 (7B)	cow 327129 (8D)
10 ml		cow 325452 (7A) cow 325452 (7C)
20 ml		cow 327129 (8C)
30 ml		cow 327129 (8A)

Numbers and capitals within brackets refer to the numbers of tables in which the results of treatments (indicated by capitals) have been given

TABLE III List of treatments of cattle with *hexachlorophene*
(An asterisk before a number indicates that hexachlorophene has been given in combination with another compound).

Dose rate (mg G-11/kg)	Mode of application				
	oral			subcutaneous	
	suspension in water	solubilized in water	solution in olive oil	solution in olive oil	solution in alcohol
5	*217561 (10D) *326539 (10E) *305786 (10C) *306493 (11A) *460288 (11B) *361084 (11C) *429951 (11D)	305786 (10B)		5104 (10A) 9511 (10A)	
10	301660 (12A)		301694 (12B)	301635 (12C) 207551 (12C)	
14	662205 (14D)		208497 (13C)		
15	5104 (10F) 9511 (10F) 301660 (12D) 301694 (12D) 301635 (12D) 208211 (13D) 368640 (13D) 208247 (13D) 209810 (14A) 452133 (14A) 779763 (14A) 369304 (14B) 321437 (14C)		31568 (13B)	208084 (13A)	
20	208489 (15A) 779768 (15A) 1531 (15B) 1556 (15B) 2927 (15B) 734 (16C) 26737 (16C)				
39					4139 (16A)
40					5111 (16B)

Numbers and capitals within brackets refer to the numbers of tables in which the results of treatments (indicated by capitals) have been given.

TABLE IV List of treatments of sheep with *hexachlorophene*

Dose rate (mg G-11/kg)	Mode of application					
	oral				subcutaneous	
	suspension in water	solution in propylene glycol	solution in olive oil	solubilized in water	solution in olive oil	solution in Tween 80
5	1596 (24A) 1597 (24A) 1598 (24A) 1117 (25A) 1133 (25A)		1083 (24C) 1181 (24C)		1545 (24B)	
10	P (25B) 1595 (25B) 1599 (25B)		1117 (25C) 1133 (25C)			
12		1117 (25D)				
15		1083 (24D) 1181 (24D) 1599 (25E) 1133 (25E) 1153 (27D) 45682 (28A) 45684 (28A) 45691 (28A) 1546 (32B)	45681 (26A) 45683 (26A) 45685 (26A) 45689 (26A) 1040 (26A) 1031 (26A) 1153 (27A) 45692 (27A) 1546 (32D)		1599 (25F) 1117 (25F) 1133 (25F) 1547 (27C) 45686 (27C) 45690 (27C) 45694 (27C) 1546 (32E)	45692 (27B) 45693 (27B)
18				63 (30A)		
19				62 (30B)		
20				45693 (27E) A–5 (29A) A–25 (29A) A–30 (29A) A–47 (29A) A–67 (29A) 1999 (29A) 45695 (33D)		
23		1117 (25G)				
30		1546 (32C)		40820 (30C) 40821 (30C) 40822 (30C) 40825 (30C) 40815 (31A) 40816 (31A) 40818 (31A) 40819 (31A) 40829 (31A) 40830 (31A) 1546 (32G)		

Numbers and capitals within brackets refer to the numbers of tables in which the results of treatments (indicated by capitals) have been given

TABLE V List of treatments of sheep with *hexachlorophene-piperazine complex*

Dose rate (mg/kg)	Mode of application	
	oral (suspension in water)	subcutaneous (suspension in olive oil)
15	1546 (32A)	
30		45687 (33A)
35		1546 (32F)
40		45695 (33B)
50		45688 (33C)

Numbers and capitals within brackets refer to the numbers of tables in which the results of treatments (indicated by capitals) have been given

TABLE VI List of treatments of cattle with "*Hetol*"
The dose rate is expressed in mg/kg of the active principle: 1,4-di(trichloromethyl)benzene

Dose rate (mg/kg)	Animal and Treatment
99	cow 326686 (19A)
136	cow 369941 (19C) cow 326539 (19C)
150	cow 326686 (19B) cow 369941 (19B) cow 326539 (19B) cow 217561 (20A) cow 325452 (20A) cow 327129 (20A) cow 420219 (20A)
225	cow K88033 (21A) cow 241792 (21A) cow 17397 (21A) cow 364024 (21A) cow 712068 (21A)

Numbers and capitals within brackets refer to the numbers of tables in which the results of treatments (indicated by capitals) have been given

EXPLANATORY NOTES CONCERNING TABLES 1 - 34

SL slaughtered

M mature *Fasciola hepatica*

I immature *F. hepatica*

\+ At least one of the daily examinations yielded one or more eggs in 3 grams of faeces, but eggs were not found within the squares of the counting slide.

— None of the daily examinations yielded eggs in 3 grams of faeces.

~ This symbol has been used a number of times for sheep when none of the daily examinations yielded eggs in both chambers (within and outside the squares) of the counting slide, and when examination for the presence or absence of eggs in 3 grams of faeces has not been carried out.

N.B.: The volume of each chamber of the counting slide is 2 ml, whereas the volume from which eggs may sediment on the surface within a square is 1 ml.

Mean counts in brackets: average of less than four daily egg counts.

The number of weeks after purchase is obtained by addition of the number mentioned at the same level as the heading "Weeks after purchase" and the numbers under that heading. Calf 7642 (Table 2) for example, was medicated 15, 18, and 24 weeks after purchase respectively; the animal died 25 weeks after purchase, and the first weekly mean count has been recorded 13 weeks after purchase. All numbers of weeks are rounded off.

Body weight: Generally the experimental animals have been weighed on the day of medication or a few days earlier.

mg/kg: milligrams per kilogram body weight.

TABLE 1 *Untreated cows:* weekly mean counts (eggs per gram of faeces), and numbers of flukes containing eggs. Each weekly count represents the average of three daily egg counts within a week. All animals were more than two years old

Cow	Weeks after purchase		Weekly mean count (eggs per gram of faeces)	Number of flukes
	egg counts	slaughtered		
2772	18	25	+	2
7718	28	30	+	4
6127	24	35	+	9
0156	24 33	33	2 2	5
2718	17	18	2	15
9587	25	26	2	30
10897	25	26	3	28
6562	17	20	5	43
7916	28	37	5	58
1893	26	29	8	17
2502	25	28	13	41
2496	26	28	13	66
2719	19	21	18	106
3771	17	19	37	175

The animals were all purchased in winter, between the 16th Dec. 1957 and the 24th Feb. 1958

TABLE 2 Weekly mean counts (eggs per gram of faeces), before and after treatment

Cattle		7642		308423		458283		361083	
Body weight (kg)		about 170–180		572		542–539		609	
Date of purchase		30.I.58		3.XI.58		6.X.58		4.XI.58	
Weeks after purchase		15		2		1		3	
—2		120		26				13	
—1		119		33		8		22	
0		105 (A)		29 (D)		24 (F)		19 (G)	
+1		142		15		7		18	
+2		112		26		15		(25)SL	
+3		108 (B)		(35)SL		7			
+4		111				12			
+5		96				17 (E)			
+6		56				19			
+7		95				17			
+8		93				(23)SL			
+9		45 (C)							
+10		113 Died[1]							
		M	I	M	I	M	I	M	I
Flukes in bile ducts and gall-bladder	living	225[2]	0	131	0	28	0	35	0
	dead		0	0	0	1	0	0	0
Flukes in liver parenchyma	living	0	0	0	1	0	1	0	2
	dead	0	0	0	0	0	0	0	0

A 1.6 g *ammonium dinitro-o-cresol*, dissolved in water, administered by stomach tube

B *n-butyryl phloroglucinate*: Monday: 3 g; Thursday: 6 g; Friday :7.7 g
The drug was dissolved in water, and administered by stomach tube

C 9 g *1-8-dihydroxy-anthraquinone ("Diaquone")*, in water, administered orally

D 10 mg *2,4,5-trichlorophenol*/kg, suspended in water (with carboxymethylcellulose as a wetting agent), administered by stomach tube

E 20 mg *2,4,5-trichlorophenol*/kg, suspended in water (with sodium laurylsulphate as a wetting agent), administered by stomach tube

F 20 mg *2,2'-methylenebis(4,6-dichlorophenol)*/kg, suspended in water (with carboxymethylcellulose as a wetting agent), administered by stomach tube

G *sodium pentachlorophenate:* Monday: about 20 mg/kg; Tuesday: 25 mg/kg; Wednesday: 25 mg/kg. The drug was dissolved in water, and administered orally

[1] Autopsy revealed pleuritis and local peritonitis
[2] These 225 flukes were probably all alive when the host died

TABLE 3 Cow 8902; body weight in period of experiment: 400–510 kg; date of purchase: 5.I.1957

Weeks after purchase	Weekly mean counts (eggs per gram of faeces)	Weeks after purchase	Weekly mean counts (eggs per gram of faeces)
25	39	43	9
26	39	44	6
27	33	45	10
28	28 (A)	46	6
29	33	47	8 (D)
30	32	48	5
31	28	49	7
32	24 (B)	50	(5) (E)
33	21 (C)	51	(5)
34	14	52	6 (F)
35	8	53	5 (G)
36	6	54	(—)
37	6	55	+ (H)
38	8	56	3
39	5	57	2 (J)
40	8	58	4 (K)
41	7	59	3
42	5	60	4

A *2-acetylamino-5-nitrothiazole* (*"Enheptin A"*): Tuesday to Saturday included: about 35 mg/kg per day, 15% premix suspended in water, administered orally

B *stannous chloride* ($SnCl_2.2H_2O$): Thursday and Friday each day an intramuscular injection of about 2 mg/kg, dissolved in distilled water (the 4% solution, which also contained 0.05% streptomycin, was milky white because of a very fine sediment of *stannous hydroxide*; it was shaken immediately before use)

C *stannous chloride:* Monday to Thursday included: each day an intramuscular injection of about 4 mg/kg (8% solution, further as under B)

D *kamala:* Monday to Thursday included: 95 mg/kg per day, suspended in water (with carboxymethylcellulose as a wetting agent), administered orally

E *kamala:* Monday and Tuesday: 125 mg/kg per day, administered as under D

F *kamala:* Monday, Tuesday and Wednesday: 167 mg/kg per day; Thursday: 146 mg/kg, administered as under D

G *phloroglucinol:* Tuesday: 20 mg/kg; Wednesday: 29 mg/kg; Thursday: 42 mg/kg, dissolved in water, administered orally

H *phloroglucinol:* Friday: 40 mg/kg, dissolved in "Solutio Petit ad injectionem" (Codex Medicamentorum Nederlandicus), injected subcutaneously

J *phloroglucinol:* Tuesday: 23 mg/kg; Wednesday: 35 mg/kg; Thursday: 50 mg/kg, dissolved in water, administered orally

K *resorcinol:* Wednesday, Thursday and Friday: 20 mg/kg per day, dissolved in water, administered orally

TABLE 4 Weekly mean counts (eggs per gram of faeces), before and after treatment

Cattle		209812	360413	360406
Body weight (kg)		577	574–559	624–621
Date of purchase		28.X.58	21.X.58	21.X.58
Weeks after purchase		2	2	2
−2		(12)	(45)	(32)
−1		21	47	25
0		2 (A)	23 (B)	13 (D)
+1		3	26	7
+2		6	27	9
+3			26 (A)	16 (C)
+4			32	2
+5			(40)SL	6
+6				(13)
			M	I
Flukes in bile ducts and gall-bladder	living		119	0
	dead		0	0
Flukes in liver parenchyma	living		0	3
	dead		0	0

A 50 mg *sodium pentachlorophenate*/kg, dissolved in water, administered by stomach tube

B 10 mg *di-n-butyl tin dilaurate*/kg, administered orally

C 20 mg *di-n-butyl tin dilaurate*/kg, administered orally

D about 39 mg *bithionol*/kg, suspended in water (with carboxymethylcellulose as a wetting agent), administered by stomach tube

TABLE 5 Calf 1115; date of birth: 10.XI.1957

Experimentally infected: 31.VII.58: 25 encysted metacercariae
13.VIII.58: 50 encysted metacercariae
8.IX.58: 200 encysted metacercariae

Weeks after last administration of cysts	Weekly mean counts (eggs per gram of faeces)	Weeks after last administration of cysts	Weekly mean counts (eggs per gram of faeces)
5	(—)*	29	(40)*
6	(+)*	30	54 (A)
7	(—)*	31	21
8	(1)*	32	19
9	(3)*	33	18
10	(3)*	34	14
11	(10)*	35	14
12	(10)*	36	18
13	(25)*	37	7
14	(20)*	38	16
15		39	15 (B)
16	(45)*	40	12
17	(50)*	41	13
18	(60)*	42	11
19	(70)*	43	+
20	(95)*	44	3
21	(70)*	45	5
22	(70)*	46	1
23	(85)*	47	3
24	(40)*	48	3
25	(95)*	49	5
26	(40)*	50	4
27	(70)*	51	4SL
28	(90)*		

Bile ducts: 4 living mature flukes
Liver parenchyma: no flukes found

A 30 mg *di-n-butyl tin dilaurate*/kg, emulsified in water with sodium laurylsulphate and Tween 80, administered by stomach tube. Body weight: 335 kg

B 400 mg *di-n-octyl tin dilaurate*/kg, emulsified in water with Tween 80, administered by stomach tube. Body weight: 374 kg

* one faecal sample examined per week

TABLE 6 Cow 2273; body weight in period of experiment: about 500–600 kg; date of purchase: 31.I.1955

Weeks after purchase	Weekly mean counts (eggs per gram of faeces)	Weeks after purchase	Weekly mean counts (eggs per gram of faeces)
17	51	45	5
18	38	46	7
19	36	47	8
20	9 (A)	48	7
21	10	49	7
22	11	50	8
23	20	51	8
24	17	52	7
25	20	53	7
26	16	54	7
27	8	55	8 (C)
28	10	56	6
29	15	57	5
30	13	58	6
31	18	59	5
32	15	60	5
33	10	61	4
34	9 (B)	62	5
35	3	63	5
36	1		
37	4	93	5
38	5	94	5
39	5	95	4
40	5	96	5
41	6	97	5 (D)
42	3	98	5
43	3	99	(5)
44	10	100	(5)
		101	(5)

A oral dose of 4 ml *carbon tetrachloride**, mixed with 6 ml liquid paraffin
B oral dose of 5 ml *carbon tetrachloride**, mixed with 7.5 ml liquid paraffin
C oral dose of 4 ml *carbon tetrachloride**, mixed with 6 ml liquid paraffin
D oral dose of 3 ml *carbon tetrachloride**

* Dutch Pharmacopoeia, 5th Ed.

TABLE 7 Cow 325452; body weight in period of experiment: about 600 kg; date of purchase: 8.VI.1959

Weeks after purchase	Weekly mean counts (eggs per gram of faeces)	Weeks after purchase	Weekly mean counts (eggs per gram of faeces)
5	49	23	17
6	61	24	12
7	68	25	9
8	44 (A)	26	10
9	48		
10	49	43	5
11	51	44	8
12	54	45	6
13	59	46	9
14	67	47	6 (C)
15	70	48	5
16	73	49	4
17	71	50	8
18	47 (B)	51	9
19	8	52	4
20	8	53	4
21	17	54	+
22	19	55	4

A 10 ml *carbon tetrachloride*[1], mixed with 10 ml liquid paraffin, injected intramuscularly

B 5 ml *carbon tetrachloride*[1], mixed with 5 ml liquid paraffin, and emulsified in water with Tween 80, administered by stomach tube

C 10 ml *carbon tetrachloride*[2], mixed with 10 ml liquid paraffin, injected intramuscularly

[1] analytical reagent grade (Union Chimique Belge, S.A.)
[2] commercial grade

TABLE 8 Cow 327129; body weight in period of experiment: 600–700 kg; date of purchase: 29.VI.1959

Weeks after purchase	Weekly mean counts (eggs per gram of faeces)	Weeks after purchase	Weekly mean counts (eggs per gram of faeces)
4	43	31	19
5	43	32	21
6	45	33	27
7	45 (A)	34	14 (C)
8	34	35	13
9	32	36	14
10	47	37	20
11	33	38	20
12	34	39	14
13	39 (B)	40	17
14	32	41	17
15	33	42	23
16	23	43	19
17	23	44	16 (D)
18	18	45	23
19	20	46	17
20	30	47	25
21	10	48	15
22	11	49	23
23	24	50	21
		51	13
		52	21

A 30 ml *carbon tetrachloride*[1], mixed with 30 ml liquid paraffin, injected intramuscularly

B 4 ml *carbon tetrachloride*[1], mixed with 4 ml liquid paraffin, administered orally

C 20 ml *carbon tetrachloride*[1], mixed with 20 ml liquid paraffin with 0.5% lidocaine, injected intramuscularly (treatment after KOVÁCS, 1959)

D 5 ml *carbon tetrachloride*[2], mixed with 5 ml liquid paraffin, injected intramuscularly

[1] analytical reagent grade (Union Chimique Belge, S.A.)

[2] commercial grade

TABLE 9 Weekly mean counts (eggs per gram of faeces), before and after administration of "*Vitan*"

Cattle	1864	4139	5111	7642	8902
Body weight (kg)	about 580	about 250	about 500	about 160	520
Date of purchase	20.I.58	21.I.58	21.I.58	30.I.58	5.I.57
Weeks after purchase	7	8	8	5	61
—3	3	70	39	673	4
—2	1	74	63	270	3
—1	1	41	43	219	4
0	1 (A)	56 (D)	50 (E)	79 (H)	3 (J)
+1	+	9	28	31	1
+2	+	10	16	146	4
+3	(+)	17	23 (F)	134	3
+4	1 (B)	13	21 (G)	990	5
+5	— (C)	11	15	638	5
+6	+	20	30	72	3
+7	—	21	25	120	4
+8	+	16	35	119	5
+9	+		27		
+10	—		33		
+11	—				
+12	—				
+13	(—)*				
+14	(—)*				
+15	(—)*				
+16	(—)*				
+17	(—)*				
+18	(+)*				
+19	(+)*				
+20	(+)*				
+21	(+)*				
+22	(+)*				
+23	(—)*				
+24	(+)**				

A 20 ml "Vitan", administered in capsules
B 20 ml "Vitan", administered by stomach tube
C 20 ml "Vitan", administered orally
D 8 ml "Vitan", administered by stomach tube
E 12 ml "Vitan", administered by stomach tube
F 16 ml "Vitan", administered by stomach tube
G 16 ml "Vitan", administered by stomach tube
H 8 ml "Vitan", administered in capsules
J 20 ml "Vitan", administered in capsules

* one faecal sample examined per week
** two faecal samples examined per week

TABLE 10 Weekly mean counts (eggs per gram of faeces), before and after treatment

Cattle	5104	9511	305786	217561	326539
Body weight (kg)	480–430	500	555–561	627	559
Date of purchase	10.VI.58	10.VI.58	6.X.58	29.VI.59	22.VI.59
Weeks after purchase	10	9	1	7	9
—3	34	26		25	48
—2	36	30		37	36
—1	74	33	93	33	53
0	52 (A)	29 (A)	91 (B)	40 (D)	54 (E)
+1	12	+	16	2	2
+2	47	7	24	6	27
+3	22	6	41	20	20
+4	20 (F)	14	35	21	14
+5	+	4	41 (C)	15	15
+6	—	2 (F)	+	14	30
+7	+ SL	—	1	11	14
+8		—	4	23	21
+9		(—)	(+)SL	19	27
+10		SL		19	30

		M	I	M	I	M	I
Flukes in bile ducts and gall-bladder	living	1	0	0	0	8	0
	dead	1	0	3	0	3	0
Flukes in liver parenchyma	living / dead	no flukes were found					

A 5 mg *hexachlorophene*/kg, dissolved in olive oil, injected subcutaneously
B 5 mg *hexachlorophene*/kg, solubilized in water with Tween 80, administered by stomach tube
C 5 mg *hexachlorophene*/kg, and 50 mg *sodium pentachlorophenate*/kg, suspended and dissolved respectively in water, administered by stomach tube
D 5 mg *hexachlorophene*/kg, suspended in water (with sodium laurylsulphate as a wetting agent), and 2 ml *carbon tetrachloride*[1], mixed with 2 ml liquid paraffin and emulsified in water, administered by stomach tube
E 5 mg *hexachlorophene*/kg, and 15 g *hexachloroethane*, both suspended in water (with sodium laurylsulphate as a wetting agent), administered by stomach tube
F 15 mg *hexachlorophene*/kg, suspended in water (with carboxymethylcellulose as a wetting agent), administered by stomach tube

[1] analytical reagent grade (Union Chimique Belge, S.A.)

TABLE 11 Weekly mean counts (eggs per gram of faeces), before and after treatment

Cattle	306493	460288	361084	429951
Body weight (kg)	625	606	563	563
Date of purchase	14.X.58	20.X.58	4.XI.58	3.XI.58
Weeks after purchase	5	1	3	4
—3	123		(28)	18
—2	66		26	45
—1	76	44	12	21
0	88 (A)	42 (B)	12 (C)	20 (D)
+1	74	1	4	9
+2	8	4	14	4
+3	11	6	5	(8)
+4		3		

A 5 mg *hexachlorophene*/kg, and 40 mg *dichlorophene*/kg, both suspended in water (with sodium laurylsulphate as a wetting agent), administered by stomach tube

B 5 mg *hexachlorophene*/kg, and 7 mg *dichlorophene*/kg (2 mol G-4 per mol G-11), suspended in water (with carboxymethylcellulose as a wetting agent), administered by stomach tube

C 5 mg *hexachlorophene*/kg, and 0.7 mg *dichlorophene*/kg (0.2 mol G-4 per mol G-11), suspended as under A, administered by stomach tube

D 5 mg *hexachlorophene*/kg, and 0.4 mg *bithionol*/kg (0.1 mol bithionol per mol G-11), suspended as under A, administered by stomach tube

TABLE 12 Weekly mean counts (eggs per gram of faeces), before and after treatment with *hexachlorophene*

Cattle		301660		301694		301635		207551
Body weight (kg)		596–610		555–560		620–624		630
Date of purchase		26.VIII.58		26.VIII.58		25.VIII.58		25.VIII.58
Weeks after purchase		1		1		1		1
—1		(68)		(15)		114		16
0		49 (A)		20 (B)		50 (C)		5 (C)
+1		1		+		2		1
+2		+		+		3		+
+3		+ (D)		+ (D)		+ (D)		(+)
+4		+		—		—		
+5		—		—		+		
+6		—		—		(+)		
+7						SL		
+8		SL		SL				
		M	I	M	I	M	I	
Flukes in bile ducts and gall-bladder	living	1	0	0	0	7	0	
	dead	1	0	3	0	1	0	
Flukes in liver parenchyma	living	0	5	0	0	0	0	
	dead	0	0	0	0	0	0	

A 10 mg G-11/kg, suspended by mixing an alcoholic solution with water, administered by stomach tube

B 10 mg G-11/kg, dissolved in olive oil, administered orally

C 10 mg G-11/kg, dissolved in olive oil, injected subcutaneously

D 15 mg G-11/kg, suspended in water (with carboxymethylcellulose as a wetting agent), administered by stomach tube

TABLE 13 Weekly mean counts (eggs per gram of faeces), before and after treatment with *hexachlorophene*

Cattle		208084		31568		208497		208211		368640		208247	
Body weight (kg)		632		570		604		574		584		612	
Date of purchase		2.IX.58		15.IX.58		16.IX.58		8.IX.58		9.IX.58		9.IX.58	
Weeks after purchase		1		1		1		1		1		1	
−1		(168)		24		(28)		29		(28)		(30)	
0		68 (A)		16 (B)		37 (C)		12 (D)		47 (D)		39 (D)	
+1		+		+		+		+		+		—	
+2		+		+		—SL		(+)SL		(+)SL		—	
+3		+		(—)								+SL	
+4		—		SL									
+5		+											
+6													
+7		SL											
		M	I	M	I	M	I	M	I	M	I	M	I
Flukes in bile ducts and gall-bladder	living	0	0	1	0	1	2	0	0	0	0	2	0
	dead	13	0	0	0	1	2	1	0	3	0	12	0
Flukes in liver parenchyma	living / dead	no flukes were found											

A 15 mg G-11/kg, dissolved in olive oil, injected subcutaneously

B 15 mg G-11/kg, dissolved in olive oil, administered orally

C Somewhat less than 15 mg G-11/kg, dissolved in olive oil, administered orally (a small quantity of the solution was lost)

D 15 mg G-11/kg, suspended in water (with carboxymethylcellulose as a wetting agent), administered by stomach tube

TABLE 14 Weekly mean counts (eggs per gram of faeces), before and after treatment with *hexachlorophene*

Cattle		209810		452133		779763		369304		321437		662205
Body weight (kg)		534		610		600		595		657		670
Date of purchase		28.X.58		1.IX.58		16.IX.58		8.VI.59		6.II.61		22.VI.59
Weeks after purchase		3		1		1		2		3		35
—3		(215)								38		27
—2		193						57		37		26
—1		164		21		(10)		39		36		33
0		156 (A)		20 (A)		6 (A)		60 (B)		31 (C)		28 (D)
+1		+		—		+		13		—		4
+2		+		+		+		+		+		+
+3		1		—		(—)		—		—		+
+4		(2)SL		+		SL		+		—		—
+5				—				+		SL		+
+6								+				
+7				SL				+				
+8								+				
+9								+				
+10								—SL				
		M	I	M	I	M	I	M	I	M	I	
Flukes in bile ducts and gall-bladder	living	1	0	0	0	0	0	1	0	0	0	
	dead	6	0	1	0	2	0	0	0	10	0	
Flukes in liver parenchyma	living / dead	no flukes were found										

A 15 mg G-11/kg, suspended in water (with carboxymethylcellulose as a wetting agent), administered by stomach tube
B about 15 mg G-11/kg, in tablets[1], disintegrated in water, administered by stomach tube
C 15 mg G-11/kg, in tablets[1], disintegrated in water, administered orally
D 14 mg G-11/kg, in tablets[2], disintegrated in water, administered by stomach tube

[1] tablets containing G-11, starch, gelatine, sodium di-octyl sulphosuccinate, and magnesium stearate
[2] tablets as under 1, but without di-octyl sulphosuccinate

TABLE 15 Weekly mean counts (eggs per gram of faeces), before and after treatment with *hexachlorophene*

Cattle	208489	779768	1531	1556	2927
Body weight (kg)	574	546	675	651	630
Date of purchase	16.IX.58	16.IX.58	3.II.59	11.II.59	25.II.59
Weeks after purchase	1	1	1	5	2
—3				113	
—2				107	(43)
—1	(30)	(67)	(80)	99	27
0	11 (A)	27 (A)	21 (B)	145 (B)	23 (B)
+1	—	+	—	13	
+2	—	—	+	2	(+)*
+3		(—)	(—)	1	(+)*
+4	SL	SL		(—)	(—)*
+5				(—)*	(—)*
+6				(+)*	(—)*
+7				(—)*	(—)*
+8				(—)*	(—)*
+9				(—)*	(+)*
+10					(+)*
+11					(+)*
+12					(+)*
+13					(+)*
+14					(—)*
+15					(—)*
+16					(—)*
+17					(—)*
+18					(—)*
+19					(+)*

		M	I	M	I
Flukes in bile ducts and gall-bladder	living	0	0	0	0
	dead	0	0	5	0
Flukes in liver parenchyma	living / dead	no flukes were found			

A 20 mg G-11/kg, suspended in water (with carboxymethylcellulose as a wetting agent), administered by stomach tube

B 20 mg G-11/kg, suspended in water (with sodium laurylsulphate as a wetting agent), administered by stomach tube

* one faecal sample examined per week

TABLE 16 Weekly mean counts (eggs per gram of faeces), before and after treatment with *hexachlorophene*

Cattle	4139	5111	734	26737
Body weight (kg)	254	536	about 516[1]	about 490[1]
Date of purchase	21.I.58	21.I.58	1.IX.59	1.IX.59
Weeks after purchase	17	19	7	7
—3	20	35	(15)	
—2	21	27	16	94
—1	16	33	9	105
0	13 (A)	17 (B)	10 (C)	119 (C)
+1	—	(—)	+	+
+2	+	(—)**	—	—
+3	(—)**	(—)**	—	—
+4	(+)	(—)**	—	—
+5	(—)**	(—)**	—	—
+6	(—)**	(+)**	—	—
+7	(—)**	(—)**	—	—
+8	(—)**	(—)**	(—)*	(—)*
+9	(—)*	(+)**	(—)*	(—)*
+10	(—)**	(—)**	(—)*	(—)*
+11	(—)**	(—)**	(—)*	(—)*
+12	(—)**	(—)**	(—)*	(—)*
+13	(—)SL	(—)**	(—)*	(—)*
+14		(—)**	(—)*	(—)*
+15		SL		
		No flukes found in the bile ducts, the gall-bladder, or the liver parenchyma.		

A 39 mg G-11/kg, dissolved in alcohol, injected subcutaneously

B 40 mg G-11/kg, dissolved in alcohol, injected subcutaneously

C about 20 mg G-11/kg, suspended in water (with sodium laurylsulphate as a wetting agent), administered orally

[1] body weight estimated from length- and heart girth measurements (GREVERS-LUXWOLDA)

* one faecal sample examined per week

** two faecal samples examined per week

TABLE 17a Weekly mean counts (eggs per gram of faeces), before and after administration of *2,2'-thiobis(3,4,6-trichlorophenol), (G-11S)*

Cattle	1744	1690	1738
Body weight (kg)	410	427	404
Date of purchase	25.XI.1958	25.XI.1958	25.XI.1958
Weeks after purchase	1	1	1
—1	(38)	(53)	(75)
0	19 (A)	36 (B)	27 (C)
+1	3	3	—
+2	9	2	—
+3	(12)	(+)	(—)

TABLE 17b Daily counts (eggs per gram of faeces) in week of treatment

Cattle	1744	1690	1738
Monday	25	30	55
Tuesday	20 (A)	65 (B)	40 (C)
Wednesday		55	35
Thursday	25	30	5
Friday	5	+	+

A 5 mg G-11S/kg, suspended in water with sodium laurylsulphate, by stomach tube
B 10 mg G-11S/kg, suspended in water with sodium laurylsulphate, by stomach tube
C 15 mg G-11S/kg, suspended in water with sodium laurylsulphate, by stomach tube

TABLE 18 Weekly mean counts (eggs per gram of faeces), before and after administration of *difluorotetrachloroethane (Freon 112)*

Cattle	217561	326539
Body weight (kg)	about 700	598
Date of purchase	29.VI.59	22.VI.59
Weeks after purchase	43	51
−3	11	16
−2	13	17
−1	11	10
0	6 (A)	5 (B)
+1	15	1
+2	7	7
+3	5	8
+4	10	6
+5	3	5
+6	11	2
+7	7	9
+8	5	13
+9	5	4
+10	5	5

A 50 g Freon 112 (about 70 mg/kg), dispersed in water with carboxymethylcellulose and sodium laurylsulphate, administered by stomach tube

B about 200 g Freon 112 (about 330 mg/kg, or 0.15 g/lb), mixed with an equal volume of liquid paraffin, given orally

TABLE 19 Weekly mean counts (eggs per gram of faeces), before and after administration of *1,4-di(trichloromethyl)benzene, ("Hetol")*

Cattle		326686		369941		326539	
Body weight (kg)		690–698		763–771		596–574	
Date of purchase		23.VI.1959		23.VI.1959		22.VI.1959	
Weeks after purchase		67		67		67	
−3		8		68		4	
−2		3		49		4	
−1		13		66		4	
0		10 (A)		58 (C)		3 (C)	
+1		+		1		+	
+2		+		2		+	
+3		4		15		+	
+4		5		26		1	
+5		11		20		2	
+6		10 (B)		19 (B)		3 (B)	
+7		—		+		+	
+8		+		+		—	
+9		2		3		—	
+10		4		6		+	
+11		1		4		(+)SL	
+12		SL		SL			
		M	I	M	I	M	I
Flukes in bile ducts and gall-bladder	living	26	0	26	0	2	0
	dead	0	0	0	0	0	0
Flukes in liver parenchyma	living / dead	no flukes were found					

A 99 mg 1,4-di(trichloromethyl)benzene/kg, (80 g "Hetol"), administered by stomach tube as a suspension of "Hetol" in water

B 150 mg 1,4-di(trichloromethyl)benzene/kg, administered by stomach tube as a suspension of "Hetol" in water

C 136 mg 1,4-di(trichloromethyl)benzene/kg, administered by stomach tube as a suspension of "Hetol" in water

TABLE 20 Weekly mean counts (eggs per gram of faeces), before and after administration of *1,4-di(trichloromethyl)benzene, ("Hetol")*

Cattle		217561		325452		327129		420219	
Body weight (kg)		738		662		792		664	
Date of purchase		29.VI.59		8.VI.59		29.VI.59		20.VII.59	
Weeks after purchase		66		69		72		69	
−3		4		+		17		79	
−2		1		+		16		75	
−1		2		+		12		75	
0		2 (A)		+ (A)		11 (A)		90 (A)	
+1		1		—		—		3	
+2		—		—		+		+	
+3		—		+		4		2	
+4		—		—		4		2	
+5		—		+		4		2	
+6		—		—		SL		SL	
+7		(+)**		(—)**					
+8		(—)**		(—)**					
+9		(—)**		(+)**					
+10		(—)**		(+)**					
+11		SL		SL					
		M	I	M	I	M	I	M	I
Flukes in bile ducts and gall-bladder	living	2	0	2	0	14	0	7	0
	dead	0	0	0	0	3	0	0	0
Flukes in liver parenchyma	living / dead	no flukes were found							

A 150 mg 1,4-di(trichloromethyl)benzene/kg, administered by stomach tube as a suspension of "Hetol" in water

** two faecal samples examined per week

TABLE 21 Weekly mean counts (eggs per gram of faeces), before and after administration of *1,4-di(trichloromethyl)benzene, ("Hetol")*

Cattle		K88033		241792		17397		364024		712068	
Body weight (kg)		522		592		625		583		642	
Date of purchase		23.I.61		24.I.61		15.V.61		15.V.61		29.V.61	
Weeks after purchase		3		3		4		4		6	
—3		124		67		23		15		23	
—2		85		53		16		17		28	
—1		104		49		20		18		20	
0		139 (A)		33 (A)		11 (A)		9 (A)		24 (A)	
+1		(+)SL		(3)SL		+		+		1	
+2						—		+		+	
+3						SL		SL		+	
+4										—	
+5										(—)SL	
		M	I	M	I	M	I	M	I	M	I
Flukes in bile ducts and gall-bladder	living	3	0	3	0	0	0	2	0	6	0
	dead	3	0	6	0	0	0	2	0	0	0
Flukes in liver parenchyma	living	0	1	0	0	0	0	0	0	0	0
	dead	0	0	0	0	0	0	0	0	0	0

A 225 mg 1,4-di(trichloromethyl)benzene/kg, administered by stomach tube as a suspension of "Hetol" in water

TABLE 22a Sheep 45695; weekly mean counts (eggs per gram of faeces), before and after oral administration of a solution of 6.0 grams of *di-n-octyl tin dichloride* in about 30 ml olive oil. Dose: 200 mg di-n-octyl tin dichloride per kg body weight

Weeks after purchase	Weekly mean counts
8	(1172)
9	1321
10	1039
11	1014
12	718 (treatment)
13	345
14	529
15	589
16	681
17	791
18	691
19	783
20	699
21	785
22	1020
23	779
24	887
25	984
26	935
27	1054

TABLE 22b Sheep 45695; daily egg counts (eggs per gram of faeces) in week of treatment (mean: 718 eggs/gram)

Day	eggs per gram
Monday	940
Tuesday	1115 (treatment)
Wednesday	395 (diarrhoea)
Thursday	815
Friday	325

TABLE 23 Sheep 479; Date of purchase: 3.XI.1956; daily egg counts (eggs per gram of faeces)

Date	Eggs per gram of faeces
3.I.1957	995
10.I.57	1150
21.I.57	730
8.II.57	600
18.II.57	880
19.II.57	650
20.II.57	800
21.II.57	600
22.II.57	675
23.II.57	1 ml *carbon tetrachloride*[1], mixed with 1.5 ml liquid paraffin, by mouth
14.III.57	45
19.III.57	80
11.IV.57	110
10.V.57	200
13.V.57	treatment as on 23.II.57
20.V.57	70
27.V.57	40
4.VI.57	125
11.VI.57	165
17.VI.57	60
18.VI.57	105
24.VI.57	160
1.VII.57	205
10.VII.57	140
17.VII.57	170

[1] Dutch Pharmacopoeia, 5th Ed.

TABLE 24 Weekly mean counts (eggs per gram of faeces), before and after treatment with *hexachlorophene*

Sheep		1596		1597		1598		1545		1083		1181	
Weeks after purchase		0		1		1		2		4		4	
−3												215	
−2								(100)		(595)			
−1				7367		(1413)		58		612		176	
0		103 (A)		4319 (A)		(150)(A)		80 (B)		(175)(C)		(148)(C)	
+1		(32)SL		8		(~)		+		(+)		(+)	
+2				(3)		(+)		23		(+)		1	
+3				4		4		15		10		3	
+4				(13)SL		(5)SL		(15)†		(23)		(5)	
+5										(30)		(18)	
+6										26		48	
+7										35		(70)	
+8										55		119	
+9										88		136	
+10										51		141	
+11										105		(213)	
+12										(105)(D)		(200)(D)	
+13										~		~	
+14										(~)SL		(~)SL	
		M	I	M	I	M	I	M	I	M	I	M	I
Flukes in bile ducts and gall-bladder	living	0	0	0	0	1	1	1?	0	0	0	0	0
	dead	0	0	4	0	0	0	4?	0	0	0	0	0
Flukes in liver parenchyma	living / dead	no flukes were found											

A 5 mg G-11/kg, suspended in water (with sodium laurylsulphate as a wetting agent), administered by stomach tube

B 5 mg G-11/kg, dissolved in olive oil, injected subcutaneously

C 5 mg G-11/kg, dissolved in olive oil, administered orally

D 15 mg G-11/kg, dissolved in propylene glycol, administered by stomach tube

† Sheep 1545 died in the night following the 27th day after treatment. Autopsy, carried out by Mr. J. P. W. M. AKKERMANS, revealed the presence of superficial petechiae in the spleen, oedema of the lungs, and a local peritonitis. Non-haemolytic streptococci were recovered from the spleen. Since the animal had died several hours before it could be examined, it was not possible to determine whether the one intact *F. hepatica*, which was found in a bile duct, had been still alive when the host died. The four remaining flukes were partially disintegrated: these had probably died at least several days earlier than the host.

TABLE 25 Weekly mean counts (eggs per gram of faeces), before and after treatment with *hexachlorophene*

Sheep		P	1595		1599		1117		1133	
Weeks after purchase		2	0		1		4		4	
—3							(288)		(240)	
—2							(500)		(362)	
—1		(358)[1]			(182)		662		274	
0		137 (B)	14 (B)		(145)(B)		713 (C)		388 (C)	
+1		~	(3)SL		5		1		15	
+2		3			79		3		30	
+3		6			52 (E)		7		55	
+4		(+)			16		13		(72)	
+5		7			107 (F)		14		67	
+6		(7)			4		40		117	
+7					5		67		134	
+8					(2)SL		67		104	
+9							35 (A)		100 (A)	
+10							18		25	
+11							61		90	
+12							43 (D)		59 (E)	
+13							9		5	
+14							43 (G)		65 (F)	
+15							56		1	
+16							58(F)		(3)	
+17							1		(~)SL	
+18							(+)			
+19							~			
+20							~			
+21							(~)SL			
			M	I	M	I	M	I	M	I
Flukes in bile ducts and gall-bladder	living		1	0	0	0	0	0	0	0
	dead		0	0	0	0	0	0	0	0
Flukes in liver parenchyma	living		no flukes were found							
	dead									

A 5 mg G-11/kg, suspended in water (with sodium laurylsulphate as a wetting agent), administered by stomach tube
B 10 mg G-11/kg, as under A
C 10 mg G-11/kg, dissolved in olive oil, administered orally
D 12 mg G-11/kg, dissolved in propylene glycol, administered by stomach tube
E 15 mg G-11/kg, dissolved in propylene glycol, administered by stomach tube
F 15 mg G-11/kg, dissolved in olive oil, injected subcutaneously
G 23 mg G-11/kg, dissolved in propylene glycol, administered by stomach tube

[1] mean of daily counts 1, 8, and 9 days after purchase

TABLE 26 Weekly mean counts (eggs per gram of faeces), before and after treatment with *hexachlorophene*

Sheep		45681		45683		45685		45689		1040		1031
Weeks after purchase		12		12		12		12		4		4
—3		573		1215		492		1620		(353)[1]		1723
—2		487		966		488		1226		683		1705
—1		472		1201		772		939		1131		2182
0		248 (A)		634 (A)		1238 (A)		626 (A)		783 (A)		956 (A)
+1		~		+		6		+		3		SL
+2		~		~		1		~		+		
+3		~		1		+		1		+		
+4		(~)SL		(~)SL		(~)SL		(3)SL		+		
+5										—		
+6										+		
+7										+		
+8										+		
+9										+		
+10										(10)SL		
		M	I	M	I	M	I	M	I	M	I	
Flukes in bile ducts and gall-bladder	living	0	0	0	0	0	0	0	0	5	0	0
	dead	0	0	0	0	0	0	0	0	0	0	35[2]
Flukes in liver parenchyma	living	0	0	0	0	0	0	0	0	0	1	tens[3]
	dead	0	0	0	0	0	0	0	0	0	0	

A 15 mg G-11/kg, dissolved in olive oil, administered orally

[1] Mean of daily counts 2, 3, and 6 days after purchase

[2] Approximately 35 dead *F. hepatica*: apart from their anterior portion they were necrotic, shrunken, and yellowish discoloured. Possibly some of these flukes had been liberated from the liver parenchyma by cutting the liver in slices.

[3] Under the liver capsule some tens of white nodules, which contained *F. hepatica*. Some of these flukes were virtually adult, and two contained eggs. The flukes from the nodules had lost their tonus, and the posterior part of some of the worms was necrotic, shrunken and discoloured. It was found impossible, however, to make certain that they were all dead.

TABLE 27 Weekly mean counts (eggs per gram of faeces), before and after treatment with *hexachlorophene*

Sheep	1153	45692	45693	1547	45686	45690	45694
Weeks after purchase	4	12	30	2	8	8	8
−3	(2640)	1051	244		(165)	(458)	(1203)
−2	(3000)	1053	255	(63)	(880)	(540)	1156
−1	1628	1657	305	(82)	(2082)	(667)	1388
0	4266 (A)	953 (A)	581 (B)	(73)(C)	436 (C)	901 (C)	942 (C)
+1	40	6	121	—	(8)	20	213
+2	3	6	5	+	1	3	+
+3	1	4	2	~	~	6	~
+4	—	9	32	(~)SL	~	5	(+)
+5	+	8	16		~	3	2
+6	+	9	10		(~)SL	(+)SL	(2)SL
+7	+	12	5				
+8	13	16	6				
+9	50	21	5				
+10	66	23	(+)				
+11	109	16	3				
+12	63 (D)	(15)	18				
+13	~	18	8				
+14	(~)SL	10	15				
+15		(23)	1 (E)				
+16		13	+				
+17		17	—				
+18		17 (B)	(+)SL				
+19		+					
+20		+					
+21		—					
+22		+					
+23		+					
+24		—					
+25		—					
+26		—					
+27		(—)SL					

		M	I	M	I	M	I	M	I	M	I	M	I	M	I
Flukes in bile ducts and gall-bladder	living	2	0	0	0	0	0	0	0	0	0	5	0	2	0
	dead	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Flukes in liver parenchyma	living	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	dead	0	1	0	0	0	0	0	0	0	2	0	0	0	0

A 15 mg G-11/kg, dissolved in olive oil, administered orally

B 15 mg G-11/kg, dissolved in Tween 80, injected subcutaneously

C 15 mg G-11/kg, dissolved in olive oil, injected subcutaneously

D 15 mg G-11/kg, dissolved in propylene glycol, administered by stomach tube

E 20 mg G-11/kg, solubilized in water with Tween 80, administered by stomach tube

TABLE 28 Weekly mean counts (eggs per gram of faeces), before and after treatment with *hexachlorophene*

Sheep		45682		45684		45691	
Weeks after purchase		8		8		8	
—3		567		678		1128	
—2		403		1166		1507	
—1		530		1203		1014	
0		234 (A)		527 (A)		452 (A)	
+1		1		8		(~)	
+2		+		~		(+)	
+3		~		~		(~)	
+4		+		~		(2)	
+5		~		~		1	
+6		SL		(~)SL		SL	
		M	I	M	I	M	I
Flukes in bile ducts and gall-bladder	living	1	0	0	0	6	0
	dead	0	0	1	0	0	0
Flukes in liver parenchyma	living	no flukes were found					
	dead						

A 15 mg G-11/kg, dissolved in propylene glycol, administered by stomach tube

TABLE 29 Weekly mean counts (eggs per gram of faeces), before and after treatment with *hexachlorophene*

Sheep		A–5		A–25		A–30		A–47		A–67		1999	
Weeks after purchase		6		6		6		6		6		6	
—3		199		186		261		687		278		177	
—2		241		159		225		528		256		169	
—1		215		172		368		572		293		178	
0		231 (A)		175 (A)		770 (A)		650 (A)		212 (A)		165 (A)	
+1		+		4		7		19		1		(2)	
+2		—		+		(+)		(—)		—		(+)	
+3		(—)SL		(—)SL		(+)SL		(—)SL		(+)SL		SL	
		M	I	M	I	M	I	M	I	M	I	M	I
Flukes in bile ducts and gall-bladder	living	0	0	0	0	2	0	0	0	0	0	0	0
	dead	0	0	0	0	0	0	0	0	0	0	0	0
Flukes in liver parenchyma	living	no flukes were found											
	dead												

A 20 mg G-11/kg, solubilized in water with Tween 80, administered by stomach tube

TABLE 30 Weekly mean counts (eggs per gram of faeces), before and after treatment with *hexachlorophene*

Sheep		63		62		40820		40821		40822		40825	
Weeks after purchase		0		0		4		4		4		4	
—3						3380		1006		1329		1310	
—2						3977		1387		1414		1468	
—1						3143		906		1454		1583	
0		65 (A)		85 (B)		(2573)(C)		841 (C)		2339 (C)		1913 (C)	
+1		11		(+)		SL		(20)SL		(80)SL		(15)SL	
+2		(—)SL		(—)SL									
		M	I	M	I	M	I	M	I	M	I	M	I
Flukes in bile ducts and gall-bladder	living	0	0	0	0	0	0	0	0	0	0	0	0
	dead	0	0	3	0	29	6	0	0	1	0	32	9
Flukes in liver parenchyma	living	0	0	0	0	0	0	40[1]		0	0	0	0
	dead	0	0	0	0	0	0			0	2	0	0

A 18 mg G-11/kg, solubilized in water with Tween 80, administered by stomach tube

B 19 mg G-11/kg, as under A

C 30 mg G-11/kg, solubilized in water with Tween 80, and preserved by orthooxyquinoline-sulphate ("Superol"), administered into the oesophagus by an internal drenching apparatus

[1] About 30 nodules were found in the liver parenchyma on cutting the liver in slices, and probably many more nodules were present throughout the liver parenchyma. Each nodule contained one or two *F. hepatica*, and a total of 40 flukes, and some fragments of disintegrated small flukes were recovered. Several of the worms seemed to be nearly mature, and one contained eggs. A few of the parasites from the nodules were partially disintegrated, but the remaining flukes had a normal appearance, apart from their being practically colourless. None of the worms showed muscular contractions in physiological saline at body temperature, and it was felt to be impossible to determine whether the intact worms were dead or alive.

TABLE 31 Weekly mean counts (eggs per gram of faeces), before and after treatment with *hexachlorophene*

Sheep		40815		40816		40818		40819		40829		40830	
Weeks after purchase		10		10		10		10		10		10	
—3		1261		465		1251		1509		1038		668	
—2		1538		452		1793		1197		459		1014	
—1		1495		603		1069		(1245)		740		894	
0		528 (A)		558 (A)		685 (A)		(723)(A)		809 (A)		1034 (A)	
+1		1		—		+		1		1		+	
+2		—		(—)		+		—		—		+	
+3		+		—		+		—		—		+	
+4		SL		SL		(—)SL		SL		SL		(—)SL	
		M	I	M	I	M	I	M	I	M	I	M	I
Flukes in bile ducts and gall-bladder	living	0	0	0	0	0	0	0	0	0	0	0	0
	dead	0	0	1	0	1	2	0	1	0	0	0	1
Flukes in liver parenchyma	living	0	0	0	0	0	0	0	0	0	0	0	0
	dead	0	37	0	3	0	18	0	87	0	0	0	4

A 30 mg G-11/kg, solubilized in water with Tween 80, and preserved by orthooxyquinolinesulphate ("Superol"), administered into the oesophagus by an internal drenching apparatus

TABLE 32 Sheep 1546

Weeks after purchase	Weekly mean counts (eggs per gram of faeces)	Weeks after purchase	Weekly mean counts (eggs per gram of faeces)
0	(95)	23	12
1	96	24	21
2	41 (A)	25	15
3	(8)	26	28
4	(13)	27	40
5	53	28	43
6	(58)	29	40
7	(60)	30	30
8	80 (B)	31	23
9	11	32	40 (F)
10	108 (C)	33	20
11	1	34	34
12	13	35	35
13	18	36	30
14	45	37	40
15	65	38	46
16	60	39	53
17	64 (D)	40	52 (G)
18	9	41	—
19	56	42	—
20	(71)	43	—
21	45 (E)	44	(—)SL
22	1		

No flukes were found either in the bile ducts and the gall-bladder or in the liver parenchyma

A 15 mg *hexachlorophene-piperazine complex*/kg, suspended in water (with sodium laurylsulphate as a wetting agent), administered by stomach tube

B 15 mg *hexachlorophene*/kg, dissolved in propylene glycol, administered by stomach tube

C 30 mg *hexachlorophene*/kg, as under B

D 15 mg *hexachlorophene*/kg, dissolved in olive oil, administered orally

E 15 mg *hexachlorophene*/kg, dissolved in olive oil, injected subcutaneously

F about 35 mg *hexachlorophene-piperazine complex*/kg, suspended in olive oil, injected subcutaneously

G 30 mg *hexachlorophene*/kg, solubilized in water with Tween 80, administered by stomach tube

TABLE 33 Weekly mean counts (eggs per gram of faeces)

Sheep		45687		45695		45688	
Weeks after purchase		15		27		22	
—3		1483		887		904	
—2		1089		984		855	
—1		1551		935		1195	
0		1285 (A)		1054 (B)		995 (C)	
+1		241		385		28	
+2		6		4		3	
+3		7		1		21	
+4		4		1		8	
+5		1		1		+	
+6		3		1		1	
+7		2		2		+	
+8		3		1		+	
+9		3		6		+	
+10		4		6		(—)	
+11		3		10		SL	
+12		4		4			
+13		4		(12)			
+14		+		(8)			
+15		+		10			
+16		+		5			
+17		(—)		(5)			
+18		SL		14 (D)			
+19				—			
+20				—			
+21				(—)SL			
		M	I	M	I	M	I
Flukes in bile ducts and gall-bladder	living	0	0	0	0	0	0
	dead	0	0	0	0	0	0
Flukes in liver parenchyma	living	no flukes were found					
	dead						

A 30 mg *hexachlorophene-piperazine complex*/kg, suspended in a 2% solution of aluminum monostearate in olive oil, injected subcutaneously

B 40 mg *hexachlorophene-piperazine complex*/kg, suspended in olive oil, injected subcutaneously

C 50 mg *hexachlorophene-piperazine complex*/kg, suspended in a 2% solution of aluminum monostearate in olive oil, injected subcutaneously

D 20 mg *hexachlorophene*/kg, solubilized in water with Tween 80, administered by stomach tube

TABLE 34 Weekly mean counts (eggs per gram of faeces), before and after treatment with "*Hetol*"

Sheep		40828		40833		40835		40817		40832		40834	
Weeks after purchase		24		29		32		42		42		42	
−3		585		319		118		822		527		244	
−2		544		474		68		672		544		202	
−1		531		434		85		869		682		235	
0		2548 (A)		329 (A)		40 (A)		991 (A)		1356 (A)		228 (A)	
+1		78		72		15		+		45		15	
+2		2		+		+		+		+		+	
+3		1		—		(+)		+		+		+	
+4		—		(—)SL		SL		—		+		+	
+5		SL						(+)SL		SL		SL	
		M	I	M	I	M	I	M	I	M	I	M	I
Flukes in bile ducts and gall-bladder	living	0	0	0	0	0	0	0	0	2	0	0	0
	dead	0	0	0	0	0	0	0	0	0	0	0	0
Flukes in liver parenchyma	living	no flukes were found											
	dead												

A 160 mg/kg of *1,4-di(trichloromethyl)benzene*, administered by stomach tube as a suspension of "Hetol" in water

TABLE 35 Symptoms of intoxication exhibited by cattle after administration of hexachlorophene in doses higher than 15 mg per kg

Cattle	Dose rate (mg/kg)	Mode of administration	Pre-treatment egg count*	Diminished appetite	Inactivity	Increased respiratory rate	Diarrhoea	Salivation	Remarks
c1300	20	P	0	—	—	—	—	—	
Y1157	20	P	0	—	—	—	—	—	
Y1217	20	P	0	—	—	—	—	—	
C208489	20	Q	30	—	—	—	—	—	
C779768	20	Q	67	—	—	—	—	—	
C734	20	R	9	—	—	—	—	—	
C26737	20	R	105	—	—	—	—	—	
c1157	30	Q	0	—	—	—	—	—	
c1519	30	R	8	+	+	—	—	—	A
c1520	30	R	+	+	+	—	—	—	A
c1521	30	R	—	+	+	—	+	+	
c2438	30	R	—	+	—	+	—	—	
Y137	30	R	2	+	—	—	—	—	
Y590	30	Q	0	+	+	—	—	—	
Y1494	30	R	+	+	+	+	—	—	
Y1495	30	R	+	+	—	—	—	—	
Y1518	30	R	+	—	—	—	—	—	
Y1526	30	R	5	+	+	+	—	—	
Y12161	30	R	5	—	—	—	—	—	
Y18551	30	R	28	—	—	—	+	—	
C209812	30	R	6	—	—	+	+	—	B
C460288	30	R	3	—	—	—	+	—	
c1217	40	Q	0	+	+	—	—	—	
c4139	39	S	16	—	+	—	—	—	
Ox 5111	40	S	33	+	—	—	—	—	
C8902	40	S	4	+	+	+	—	+	C

* *Fasciola* eggs per gram of faeces; mean of three to five daily egg counts within a week. Egg count indicated as 0 means: faeces not examined because the animal was housed since birth at the institute.

Letters before the numbers of the animals: c = calf; Y = yearling; C = cow.
Mode of administration:

P solution of G-11 in propylene glycol, mixed with water immediately before administration by stomach tube

Q suspension of G-11 in water, prepared with carboxymethylcellulose as a wetting agent, administered by stomach tube

R suspension of G-11 in water, prepared with sodium laurylsulphate as a wetting agent, administered by stomach tube

S solution of G-11 in ethyl alcohol, injected subcutaneously

Remark A marked hypoexcitability the 2nd day after treatment

Remark B Cow 209812 had received an oral dose of sodium pentachlorophenate (50 mg/kg) three weeks earlier

Remark C Apart from the mentioned symptoms: extensive oedema around the sites of injection, fever, and sweating. The animal died six days after treatment

TABLE 36 Symptoms of intoxication exhibited by cattle after treatment with hexachlorophene (20 mg/kg) close to parturition. A watery suspension of the drug, prepared with sodium laurylsulphate as a wetting agent, was administered by stomach tube

Cow	Medication		Pre-treatment egg count*	Diminished appetite	Inactivity	Increased respiratory rate	Diarrhoea	Remarks
	Days before calving	Days after calving						
1531	18		80	+	+	+	+	A
		3		+	—	+	+	B
2110	43		+	—	—	—	—	
		2		—	—	—	—	
1556	3		99	—	—	—	—	
1566	34		2	—	—	—	+	
		3		—	—	—	—	
2927	34		27	—	—	—	—	
		8		—	—	—	—	
1583	5		12	—	—	+	—	C
4619	15		3	—	—	—	—	
		1		—	—	—	—	
15555	6		—	—	—	—	—	D
		8		+	—	—	—	

* *Fasciola* eggs per gram of faeces; mean of three to five daily egg counts within a week

Remark A No rumination on the 2nd day after treatment

Remark B The placenta was retained until the day of treatment. The animal showed fever (rectal temperature 41°C) the 2nd day after treatment, and intramuscular injections of procaine penicillin G and streptomycin were administered the 2nd and 3rd day after treatment with hexachlorophene. The temperature was again normal the 3rd day after medication with G-11

Remark C The respiratory rate was only slightly increased the first day after treatment

Remark D The calf of cow 15555 was somewhat weak during the first two days of its life

TABLE 37 Milk yield of cows in kg/day, before and after administration of 15 mg hexachlorophene per kg body weight by the oral route

Days after treatment	Cow								
	9511	5104	301635	301660	301694	368640	208247	779763	208497
—7	9½		8	12½	7½				
—6	9½	8	8½	13	7	11	10½		
—5	9½	8½	7	12½	6½	9½	9½	7½	
—4	9½	8½	8	11	9	10½	10	7	
—3	9½	8½	8	11	7	10	11½	7	4
—2	9½	9	8½	11	7	10½	11	8½	4½
—1	10	9	8½	10½	7	10	10	11	4
0	9½	8½	10	12	7	9½	10½	8	5
+1	8½	8½	9	11	7	10	10	9½	3½
+2	7½	8½	8	11½	7	9½	9½	8½	4
+3	7½	8½	8½	10	3½	9½	10	7	3
+4	8	9	8	11	M 3½	9	9½	8	3
+5	8½	9½	8½	11		10½	11	8½	3½
+6	8½	9	8	11½	4½	11	11	9	4½
+7	9	10	9	10½	5	10½	10	9	2½
+8	9	9½	9	10½	5½	10	10½	8½	3½
+9	9	9	8½	11	6	9	11	8½	3½
+10	9	8½	8½	10	6	8½	H 8	9	4½
+11	9	8½	8	11	6½	H 7½	10½	10	3½
+12	9	9	M	10	6½	10½	10½	9½	4
+13	9	9½	7	10½	6½	9½	10½	9½	4
+14	9	10	6½	10	6½		11	9	5
Pre-treatment egg count*	4	22	3	+	+	28	30	10	28

M mastitis diagnosed by Dr. TH. S. ZWANENBURG

H cow in heat

Mode of administration:
Cow 208497: somewhat less than 15 mg G-11/kg, dissolved in olive oil, administered orally (a small quantity of the solution was lost)
The other animals received by stomach tube a suspension of hexachlorophene in water, with carboxymethylcellulose as a wetting agent

* *Fasciola* eggs per gram of faeces; mean of three to five daily egg counts within a week

TABLE 38 Milk yield of cows in kg/day, before and after administration of 20 or 30 mg hexachlorophene per kg body weight by the oral route. (Dosage rate within brackets)

Days after treatment	Cow				
	208489	0137	1494	1495	209812
—7					8
—6			2		8½
—5	7	4½	4	5	8½
—4	10	6½	4	7	8½
—3	10½	7	4½	6½	8½
—2	11	7	4½	7	8
—1	7	7½	6	6½	H 8½
0	7(20)	8(30)	5(30)	8(30)	7(30)
+1	6	7½	4½	7½	8
+2	5½	6½	3	3½	6½
+3	5½	6	3	4	7½
+4	6	7½	4½	6	8
+5	7½	8½	4	7	8
+6	6½	8½	4	6½	9½
+7	8	9	5	7½	8½
+8	7½	8	6	7½	8½
+9	8½	9	5½	7½	9
+10	7½	9	5½	8	8½
+11	7	9	5	7½	8½
+12	7½	8	5½	7	8
+13	7½	9	4½	7½	7
+14	6½	8	5½	6½	7½
Pre-treatment egg count*	30	2	+	+	6

H: cow in heat

Mode of administration:
A suspension of hexachlorophene in water, prepared with sodium laurylsulphate as a wetting agent, was administered by stomach tube. The suspension for cow 208489, however, was prepared with carboxymethylcellulose

* *Fasciola* eggs per gram of faeces; mean of three to five daily egg counts within a week

TABLE 39 Milk yield of cows in kg/day, before and after administration of G-11S by the oral route (Dose rate within brackets)

Days after treatment	Cow		
	1744	1690	1738
—5	5½	5	5½
—4	6	4½	5½
—3	5½	4½	5
—2	5½	4	5½
—1	5½	5	5
0	5(5)	4½(10)	5(15)
+1	3½	4½	4
+2	4½	4½	5
+3	6	4½	4½
+4	6	4½	6
+5	5	4½	5½
+6	5½	5½	5½
+7	6½	5	5½
+8	6½	5	5½
+9	6	4½	5
+10	5½	4½	5
+11	6	4½	5
+12	6½	5	5
+13	5½	4½	5½
+14	5½	4½	5½

SPECIFICATION OF THE EMPLOYED DRUGS AND PREPARATIONS

2-Acetylamino-5-nitrothiazole: as "Enheptin A", 15% premix, purchased from "Brocades"[1].
Alkyl tin compounds: purchased from Organisch Chemisch Instituut T.N.O., Utrecht.
Aluminum monostearate: Aluminii Stearas-Mono, purchased from "Brocades". Heavy metals: Pb, max. 0.005%; As, max. 0.0005%.
N-Butyryl phloroglucinate: synthesized by Mr. TH. P. DEKKER.
Carbon tetrachloride: vide Tables 6, 7, 8 and 23.
Chlorhexidine hydrochloride: vide pp. 51-52.
Chloroquine sulphate: as "Nivaquine" tablets, Specia.
1,2-Difluoro-1,1,2,2-tetrachloroethane: The employed Freon 112 melted at 24.5°C, and boiled at 91.0°C (Dr. G. A. VAN KLINKENBERG). According to GREENBERG & LESTER (1950) the drug melts at 24.65°C and boils at 92.8°C.
1,8-Dihydroxy-anthraquinone: as "Diaquone", Imperial Chemical (Pharmaceuticals) Ltd., Manchester, England.
Dinitro-o-cresol: commercial pesticide, origin unknown. Ammonium salt of DNC: as "Trifocide", N.V. Vondelingenplaat, Rotterdam.
"Felamine": manufactured by Sandoz Ltd., Basel, Switzerland.
Hexachloroethane: as "Avlothane", Imperial Chemical (Pharmaceuticals) Ltd., Manchester, England.
Hygromycin B: as "Hygromix", which contains 2.4 g Hygromycin B per lb, Eli Lilly and Co., Indianapolis, U.S.A.
Kamala: Glandulae Rottlerae, C.M.N.[2], and Rottlerae Glandula, Ph. Ned. V[3], both purchased from "Brocades".
Lidocaine: prepared from Lidocaine hydrochloride by Miss E. G. HOSKAM.
Lidocaine hydrochloride: Lignocaine hydrochloride B.P., Ned. Cocaine Fabriek N.V.
Menthol: Mentholum (Syntheticum), purchased from "Brocades".
2,2'-Methylenebis (4-chlorophenol): A part of the employed dichlorophene was purchased from L. Light & Co., Ltd., Poyle, Colnbrook, Bucks., England. Dichlorophene was also extracted from "Dicestal" tablets, May & Baker Ltd., Dagenham, England; the melting point of the obtained product was 159–160°C (Dr. G. A. VAN KLINKENBERG).
2,2'-Methylenebis (4,6-dichlorophenol): provided by Givaudan-Delawanna, Inc., New York, U.S.A.
2,2'-Methylenebis (3,4,6-trichlorophenol): hexachlorophene purchased from L. Light & Co, England. The melting point of the product was 160–161°C (Mr. TH. P. DEKKER and Dr. G. A. VAN KLINKENBERG). Only the solution of hexachlorophene in propylene glycol has been provided by "Brocades".
Olive oil: Oleum Olivae, Ph. Ned. V,s.g.: about 0.915, purchased from "Brocades".
Orthooxyquinoline sulphate: Oxychinolini Sulfas, Ph. Ned. VI, $(C_9H_7ON)_2.H_2SO_4$, purchased from "Brocades".
Phloroglucinol: purchased from "Brocades", melting point 210°C.
Piperazine hexahydrate: Piperazinum, C.M.N., purchased from "Brocades".
Procaine hydrochloride: as "Novocain", Farbwerke Hoechst AG.
Resorcinol: origin unknown.
Sodium lauryl sulphate: Natrii Laurylsulfas, Ph. Ned. V, purchased from "Brocades".
Sodium pentachlorophenate: purchased from "Brocades".
Stannous chloride: origin unknown.
Stannous oxide: Oxydum Stannicum album, purchased from "Brocades". An analysis by Miss E. G. HOSKAM showed that the product contained As: 324 mg/kg, and Pb: 38.7 mg/kg.
Sunflower-seed oil: Oleum Helianthi seminis, purchased from "Brocades".

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[1] "Brocades": N.V. Koninklijke Pharmaceutische Fabrieken, v/h Brocades-Stheeman & Pharmacia.
[2] C. M. N.: Codex Medicamentorum Nederlandicum.
[3] Ph. Ned. V: Pharmacopoea Nederlandica, 5th Ed.

2,2'-Thiobis (4,6-dichlorophenol): The employed bithionol melted at 187°C (Dr. G. A. VAN KLINKENBERG). According to the Monsanto Chemical Company (1955) the melting point of Bithionol U.S.P. is 187–188°C.
2,2'-Thiobis (3,4,6-trichlorophenol): provided by Sindar Corporation, New York, U.S.A.
T-pol 410: 21% solution, provided by Shell Nederland N.V.
2,4,5-Trichlorophenol: purchased from L. Light & Co.
Tween 80: purchased from Totte & Cie N.V. ,Rotterdam.

SUSPENSION OF HEXACHLOROPHENE IN WATER

As hexachlorophene floats on water, and is not easily suspended, wetting agents had to be used. In the initial experiments the amount of hexachlorophene, which was required to treat an animal, was thoroughly mixed with a sufficient volume of a 2.5% solution of carboxymethylcellulose (Cellofas B, I.C.I.) with mortar and pestle so as to obtain a homogenous paste-like consistency. Gradually more water was added, and mixing was continued. Finally a satisfactory suspension of hexachlorophene was obtained. Most suspensions, however, have been obtained in the following way. The amount of hexachlorophene, which was required to treat an animal, about 400 mg of sodium lauryl sulphate and about 15 ml water, were mixed in a beaker with a spoon. Very soon the hexachlorophene was completely wetted, and more water was added.

SOLUTION OF HEXACHLOROPHENE IN ETHYL ALCOHOL

Concentration: 100 mg/ml.

SOLUTION OF HEXACHLOROPHENE IN OLIVE OIL

Concentration: 100 mg/ml. Sterilized at 150°C for one hour.

SOLUTION OF HEXACHLOROPHENE IN PROPYLENE GLYCOL

The 10% solution of hexachlorophene in 1,2-propylene glycol was provided by "Brocades".

SOLUTION OF HEXACHLOROPHENE IN TWEEN 80

Concentration: 100 mg/ml.

SOLUBILIZED HEXACHLOROPHENE

20 grams of hexachlorophene were dissolved in 200 grams of Tween 80 at about 50°C under constant or periodic stirring. After cooling to room temperature about 700 ml of distilled water was added, and the mixture was stirred long enough to obtain a clear fluid. After addition of the water heating had to be avoided, otherwise hexachlorophene partially precipitated. It required a long period for the Tween 80 to dissolve completely, and the undissolved material tended to adhere to the vessel wall. By standing overnight the stirring time could be shortened. Finally more distilled water was added so as to bring the solution to one liter.

After preservation by 5 g of orthooxyquinoline sulphate per liter, the solution could be stored in well stoppered bottles, and no deterioration occurred.

SUSPENSION OF HEXACHLOROPHENE-PIPERAZINE COMPLEX IN OLIVE OIL

Concentration: 1 g per 3 ml. Sterilization at 150°C for one hour. Prior to administration the suspension was thoroughly stirred.

In some instances the suspension also contained aluminium monostearate. This compound, in powder form, was dissolved in the olive oil by heating at about 155°C, prior to the addition of the hexachlorophene-piperazine complex.

PREPARATION OF THE HEXACHLOROPHENE-PIPERAZINE COMPLEX

As hexachlorophene is a weak acid, it was considered that it might be useful to prepare a complex of this drug and the base piperazine, which is a well known anthelmintic. It was found that mixing of

a solution of hexachlorophene in alcohol and a solution of piperazine resulted in the formation of a white precipitate, which appeared to be virtually insoluble in water, alcohol and olive oil.

The preparation of the complex was further worked out by Mr. Th. P. DEKKER, who developed the following method.

One mol (406.92 g) of hexachlorophene is dissolved in dehydrated alcohol, preferably to about saturation. One mol (194.23 g) of piperazine hexahydrate is also dissolved in dehydrated alcohol. Both solutions are mixed, and the formed precipitate is separated from the alcohol, and it is washed with ethanol and distilled water by means of a Büchner funnel. The product is dried, and ground to a fine powder with mortar and pestle. The complex was found by Mr. DEKKER to be soluble in a solution of sodium or potassium hydroxyde, but difficultly soluble in alcohol, ether, and water. The melting point was found by Dr. G. A. VAN KLINKENBERG to be 224–227°C, whereas the melting points of hexachlorophene and piperazine are 164–165°C and 104°C respectively (H. M. RAUEN: Biochemisches Taschenbuch, Berlin, 1956). Probably two molecules of hexachlorophene and two molecules of piperazine participate in the formation of the complex, which appeared to contain no water of crystallization.

HEXACHLOROPHENE TABLETS

These were manufactured from hexachlorophene (L. Light & Co, Ltd.) by Mr. E. J. E. MEYER, Pharmaceutisch-Chemisch Laboratorium Lansberg & Zn. N.V., Rotterdam.

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