

Anthelmintic Tests, Chiefly with Tetrachlor- ethylene, for the Removal of the Hookworm, *Gaigeria pachyscelis* from Infested Sheep, with Observations on the Effects of this Drug on Other Parasitic Nematodes.

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DURING the course of investigations by one of us (R. J. O.) on the life history, mode of spread and prevention of the sheep hookworm—*Gaigeria pachyscelis*—the writers have, when material became available, tried various drugs in an attempt to find a suitable medicant for the removal of this parasite from infested sheep. The drugs mainly experimented with were chemically pure Carbon Tetrachloride and Tetrachlorethylene, while n-Butyl-Chloride, Extract of Pyrethrum and other chemicals were also tried. The two first-named drugs have, since the researches of Hall and Shillinger, etc., been extensively used in the treatment of humans and dogs infected with hookworms, the former having been found by these workers and by Daubney (1930) to be also very effective in the treatment of sheep infested with the hookworm *Bunostomum trigonocephalum*. n-Butyl-chloride was tested by Wright and Schaffer (1932) and found to be 84 per cent. effective for the removal of hookworms from dogs. Pyrethrum extract was tried because of its known lethal action on cold blooded animals and its harmlessness on warm blooded animals, and in view of the favourable reports on its efficacy against hookworms in dogs by other authors.

In the first series of tests 18 adult Persian sheep were used, all of which had by faecal examination been found to carry a heavy infection of *Gaigeria pachyscelis*. These sheep had been bought from Mr. V. Theophilus, Pudimoe, where they had acquired a natural infection on his farm. Of these sheep ten were dosed with

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tetrachlorethylene in an equal volume of meal water, six were dosed with carbon tetrachloride in an equal volume of meal water and two were kept as controls. The following table gives the results:—

TABLE I.

Number of Sheep.	Drug.	Quantity.	Worms passed during next 3 days.	Eggs in faeces 12 days after drug.*	Remarks.
36429....	C ₂ Cl ₄	3 c.c.	—	††††	Died 13 days after dosing. 124 living <i>Gaigeria</i> collected.
36440....	„	„	—	††	—
36437....	„	„	9	††††	—
36442....	„	4 c.c.	1	†††	Died 14 days after dosing. 123 living <i>Gaigeria</i> collected.
36426....	„	„	24	†††	—
36427....	„	5 c.c.	—	—	—
36441....	„	„	—	†††	Died night after dosing. 77 live <i>Gaigeria</i> collected.
36428....	„	„	—	††††	—
36435....	„	„	—	†††	—
36444....	„	10 c.c.	—	††††	—
36438....	CCl ₄	3 c.c.	—	††††	—
36433....	„	„	—	†††	—
36425....	„	4 c.c.	—	†††	—
36436....	„	„	—	††††	Died 6 days after treatment. Worms not collected.
36431....	„	5 c.c.	—	†††	—
36434....	„	„	—	††††	—
36432....	„ Untreated.	„	—	††††	—
36443....	„	„	—	†††	—

* † Few eggs present (1-10 on slide).
 †† Fair number of eggs present (11-50 on slide).
 ††† Many eggs present (51-100 on slide).
 †††† Numerous eggs present (over 100 eggs on slide).

Bags were tied on the sheep after dosing in order to collect all the faeces passed; the faeces were carefully washed and sieved and all the worms passed during the following three days were collected and counted. In order to determine the presence of eggs in the faeces, about 25 to 30 grams of fresh faeces were taken from each sheep either direct from the rectum or from the bag. These were then well broken up in tap water and then passed through several sieves to retain the coarser materials. Each was then placed in an Erlenmeyer flask and allowed to settle. The supernatant liquid was then carefully poured off and the remaining sediment was now mixed with an equal volume of glycerine and well shaken. It was then centrifuged in a tube 9 cm. deep and 3 cm. diameter, for about 3 minutes at about 1,000 revolutions per minute, when the eggs rose to the top of the liquid; these were then picked up with a flat bottomed glass rod about 1 cm. diameter and several (4 to 5) drops were transferred to a slide and examined microscopically. As the *Gaigeria* eggs are much larger and darker than those of the common round worms of sheep (*Haemonchus contortus*, *Trichostrongylus spp.* and *Oesophagostomum columbianum*) they could easily be recognised.

From Table I it will be seen that doses of 3 to 5 c.c. of Carbon tetrachloride had no effect on the removal of the worms; doses of 3 to 5 c.c. and 10 c.c. Tetrachlorethylene also had very little effect, although three of the sheep did pass out some worms, they nevertheless still retained a good infection; three sheep which died from one to fourteen days after dosing showed living hookworms on post-mortem, 124, 23 and 77 worms being collected respectively. The sheep which had received 10 c.c. passed no worms and still remained heavily infected. Sheep 36436 treated with carbon tetrachloride died six days after treatment and through a misunderstanding the worms were not collected. The two controls also passed no worms and remained heavily infested.

Previous to treatment the sheep had free access to hay and broken maize, and a week prior to treatment they daily received about 2 pounds of a 3:1 mixture of bone meal and salt, while maize was removed. Food or water was not kept away from them before or after treatment.

Three weeks after the above treatment six of the above sheep were again treated with Carbon tetrachloride and Tetrachlorethylene, and five with a mixture of equal parts of these drugs and two were kept as controls.

The following table gives the results:—

TABLE II.

Number of Sheep.	Drug.	Quantity.	Worms passed during following 3 days.	Eggs in faeces 10 days after dosing.	Remarks.
36435....	C ₂ Cl ₄	6 c.c.	—	+++	Killed following day. 39 living Gaigeria collected.
36444....	—	++++	
36438....	1	++++	
36437....	CCl ₄	..	—	+++	
36425....	1	+++	
36431....	—	+++	
36441....	CCl ₄ and C ₂ Cl ₄	..	—	+++	
36428....	4	++++	Died 2 days later. 167 living Gaigeria collected.
36433....	—	+++	Died 2 days later. 65 living Gaigeria collected.
36434....	..	8 c.c.	3	+++	
36426....	—	+++	
36432....	Untreated control.	..	—	++++	
36443....	—	+++	

Except for the last two sheep treated, the dose was in all cases mixed with 12 c.c. meal water; in the last two cases only 10 c.c. meal water was added. Since the first treatment all the sheep had free access to hay and the salt and bone-meal mixture, also they were not kept away from food or water before and after dosing. They were all in a very poor condition.

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Table II shows that 6 c.c. of Carbon tetrachloride or Tetrachlorethylene in meal water had very little or no effect on the removal of the hookworms, two worms only being removed and all the sheep still remaining heavily infested as shown by egg flotation. One of the sheep which was very weak was killed the following day, and on post-mortem 39 living *Gaigeria* were found adhering to the mucous membrane. Of the 5 sheep which received 6 and 8 c.c. of a mixture of equal amounts of these two drugs 2 sheep passed 4 and 3 hookworms respectively during the following 3 days. Two of them which died two days after treatment still had 167 and 65 living *Gaigeria* respectively, some of which were lying free in the lumen of the intestine. The two controls passed no worms and still remained heavily infected. It would thus appear that a mixture of these drugs given in 6 and 8 c.c. doses is not effective for the removal of *Gaigeria* hookworms.

As the sheep were in a very poor condition they were from now on fed on hay and a liberal supply of crushed maize plus bone-meal and salt. They picked up gradually in condition, and during the course of the next seven weeks a number gradually lost most of their infection and two eventually became free of hookworms. Centrifuge egg flotation determinations after 6 and 7 weeks on this diet and faecal culture after 6 weeks gave the results shown in the following table:—

TABLE III.

Number of Sheep.	Gaigeria eggs after 6 weeks.	Gaigeria eggs after 7 weeks.	Culture.	G.	O.c.	H.c.	Tr.	S.p.*
36425.....	—	—	—	—	—	—	—	—
36426.....	†	†	W	20	34	—	14	32
36431.....	†	†	—	—	—	—	—	—
36432.....	†††	††	M	79	5	—	12	4
36434.....	†	—	M	16	22	—	55	7
36437.....	†††	†††	M	12	—	—	35	53
36438.....	†††	†††	M	62	10	—	22	6
36441.....	††	††	W	30	14	—	56	—
36443.....	††	††	M	50	10	—	30	10
36444.....	††	††	M	47	1	—	52	—

* W=weak culture; M=medium culture. These refer to the density of the larvae on the sides of the bottle 8-10 days after culture.

G=*Gaigeria*; O.c. *Oesophagostomum*; H.c.=*Haemonchus*; Tr.=*Trichostrongylus*; S.p.=*Strongyloides*. The numbers give the percentage of the different kinds in the first 100 larvae counted.

For some inexplicable reason no larvae developed in the culture of faeces from sheep 36431, although eggs were present.

When comparing these intensities of hookworm infection, as revealed by centrifuge egg flotation determination, with those of the same sheep seven weeks previously we find that two sheep have entirely lost their infection (††† to —), two have lost two-thirds of

their infection (+++ to +), two have lost half their infection (+++ to ++), two have lost one-third of their infection (+++ to +), one has lost a quarter of its infection (+++ to ++) and one has retained its whole infection.

These sheep had now been in this experiment for four months and during this period they had been kept in a dry pen and were consequently not exposed to reinfection. The infection they still retained was that which they had brought with them. The diminution of this infection may thus be ascribed to either of two causes, firstly that many of the parasites had already reached the end of their span of life and thus naturally died, only the younger hookworms remaining; or secondly by better feed the constitution of the sheep was improved with the result that they were able to rid themselves naturally from a considerable portion of their infection. It is a fact often seen in the field that where the diet of sheep is improved, the sheep, with the improvement of their condition, tend to rid themselves of a large proportion of their worm burden. In the above case both the above-mentioned causes may have been responsible, but the writers are inclined to the view that the better diet played the principal role.

As the sheep appeared to be rapidly losing their infection and as the treatments with Carbon tetrachloride and Tetrachlorethylene had given such disappointing results it was decided to dose six of the still infected sheep with 1 to 5 c.c. of Stafford Allen's extract of Pyrethrum M.225, one sheep with 15 c.c. C_2Cl_4 and to keep one sheep as a control. In all cases the dose was mixed with 20 c.c. of distilled water and was administered by stomach tube. The results are set out in the following table:—

TABLE IV.

Number of Sheep.	Drug.	Amount.	Number of worms passed during 3 days following.	Eggs in faeces at treatment.	Eggs in faeces 10 days after treatment.
36443.....	Ext. Pyr.	1 c.c.	—	++	++
36437.....	"	"	—	++	++
36440.....	"	3 c.c.	—	+	—
36441.....	"	"	—	++	+
36431.....	"	5 c.c.	—	+	+
36438.....	"	"	—	+++	+++
36444.....	C_2Cl_4	15 c.c.	3	++	++
36432.....	Untreated Control.		—	++	++

From this table it is evident that the intensity of worm infestation remained about the same before and after treatment with Extract of Pyrethrum; it is true that after 10 days one of the sheep (36440) which had received 3 c.c. had lost its infection (+ to —) and the other (36441) which had received the same dose had its infection halved (++ to +); no worms, however, were passed during the ensuing three days after treatment so that the loss probably took place

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subsequently and before the tenth day after treatment; this loss was probably due to natural causes. It thus appears legitimate to conclude that a dose of 1 to 5 c.c. of Extract of Pyrethrum in water, dosed direct into the rumen has no effect on the expulsion of the *Gaigeria* hookworm.

The sheep which had received 15 c.c. of C_2Cl_4 in water direct into the rumen passed 3 hookworms, but the infestation remained the same. The control sheep showed no change.

As the hookworm infestation of these sheep had now become rather low it was decided to reinfest them artificially. Unfortunately during this interval several of these sheep died. The remaining few were not further experimented with except one which was very weak. This sheep was dosed with 10 c.c. C_2Cl_4 in 10 c.c. liquid paraffin and died two days later. No worms were passed and at post-mortem 334 living *Gaigeria* were found still adhering to the small intestine and eleven were free in the large intestine, of which seven were still alive. The other sheep were set aside in order that cultures may be made from them to infect fresh sheep.

While a stock of infested sheep was being prepared, the method of administering drugs into the abomasum of sheep, after a preliminary dose of copper sulphate solution, became known (Ross, 1934; Mönning and Quin, 1935), and it was decided to try this method in future experiments, again using Tetrachlorethylene and also n-Butyl-Chloride. Nine sheep were eventually obtained which showed a moderate infestation by the egg flotation method. They were divided into three groups of three each; one group received 5 c.c. C_2Cl_4 in 10 c.c. liquid paraffin, the other 10 c.c. C_2Cl_4 in 20 c.c. liquid paraffin and the third group 10 c.c. n-Butyl-Chloride in 20 c.c. liquid paraffin. Immediately prior to dosing each sheep was drenched with about 10 c.c. of a 2 per cent. copper sulphate solution. The sheep were not specially prepared, neither was food or water kept away from them before or after dosing. The results of this treatment are shown in the following table:—

TABLE V.

Number of Sheep.	Drug.	Amount.	Eggs in faeces at treatment.	Eggs in faeces 10 days after treatment.	Worms passed during following 3 days.
39173.....	C_2Cl_4	5 c.c.	+++	+++	—
39588.....	"	"	++	++	—
39998.....	"	"	++	++	—
39724.....	"	10 c.c.	++	—	28
39996.....	"	"	++	—	19
40009.....	"	"	++	—	23
39184.....	n. But. Chl.	"	++	+	5
39477.....	"	"	++	—	17
39731.....	"	"	++	++	—

Twenty days after treatment the faeces from the three sheep which had been treated with 10 c.c. Tetrachlorethylene were again examined and were again found negative for hookworm eggs.

The results of this dosing appear to show that when dosed immediately after drenching with copper sulphate solution a dose of 5 c.c. Tetrachlorethylene is not effective for the removal of *Gaigeria* hookworms; a dose of 10 c.c. n-Butyl-Chloride is about 50 per cent. effective and a dose of 10 c.c. Tetrachlorethylene is very effective. In order further to test the effectiveness of this drug the four sheep which had passed no worms in the above test were each dosed with 10 c.c. Tetrachlorethylene in 20 c.c. liquid paraffin immediately after drenching with a weak copper sulphate solution with the following results.

TABLE VI.

Number of Sheep.	Drug.	Amount.	Eggs in faeces at treatment.	Eggs in faeces 10 days after treatment.	Eggs in faeces 20 days after treatment.
39173.....	C ₂ Cl ₄	10 c.c.	+++	—	—
39588.....	„	„	++	++	++
39998.....	„	„	++	—	—
39731.....	„	„	++	++	++

The worms passed were unfortunately not collected in these cases.

This result, although not so promising as the previous one, shows that in two sheep all the hookworms were removed while in the remaining two the hookworm burden was not reduced. In these two tests we thus have five sheep out of seven freed of their hookworms. What is especially striking is that these worms were either entirely cleared or not at all. This leads one to suspect that in the five cleared sheep the drug passed direct into the abomasum and was thus able very soon to reach the hookworms in the intestine, whereas the drug passed into the rumen in the other two sheep, and consequently was not able to come in contact with the hookworms immediately and in concentrated form. These sheep also carried a fairly heavy burden of wireworms and nodular worms and it was striking that in the five cleaned sheep the number of strongyle eggs was also reduced.

Having obtained these highly promising results with the dosing of Tetrachlorethylene immediately after drenching with a 2 per cent. solution of Copper sulphate, an opportunity was awaited to try out this method of treatment on a large scale. This opportunity soon occurred in that Mr. Theophilus, who had been suffering very extensive losses in his Persian sheep due to this hookworm, offered to place his entire flock of about 1,000 sheep at our disposal if we wished to carry out any tests. This offer was accepted and one of us (R. J. O.) proceeded to his farm in the Vryburg district of the Cape Province. His adult sheep, about 480 in number, were found to be in a very bad state, very thin and very anaemic and a large percentage of them showed marked swellings under the jaw. Six full

grown ewes of these were taken and an examination of their faeces, taken from the rectum, showed that they were very heavily infested with the *Gaigeria* hookworm and also with strongyloids. They were then dosed with 10 c.c. Tetrachlorethylene mixed in an equal volume of a light neutral mineral oil after drenching with 10 c.c. of a 2 per cent. copper sulphate solution. Two days after they were slaughtered with the following results:—

TABLE VII.

No. of Sheep.	Drug.	Amount.	Hookw.	H.c.	Tr.	O.c.	Remarks.
1....	C ₂ Cl ₄	10 c.c.	—	—	—	Numerous	All living.
2....	Many	Numerous	Numerous
3....	—	—	—
4....	Numerous	Many	Fair No.
5....	1 (detached in s. int.)	—	—
6....	Numerous	Many	Many	Many	..

The above test showed that drenching as above cleaned half of the sheep of their hookworms, whereas the remaining three still remained heavily infested. In the three cleaned sheep wireworm and trichostrongyles were also absent, whereas in the remaining three both these worms were present. All six sheep were still heavily infested with nodular worms. It would thus appear from this test that where the drug is able to pass direct into the abomasum it kills not only hookworms but also wireworms and trichostrongyles. In the three sheep which remained infested the drug most probably passed direct into the rumen.

The sheep were dosed in the cool of the evening directly from the veld. After dosing, which was done by means of a suitable syringe with a long bent nozzle and squirting the drug gently down the side of the cheek, all the sheep showed marked reactions; all became somewhat giddy and sluggish and three of them developed marked tympany of the rumen which had eventually to be relieved by puncturing with a trocar. Next morning, however, all six were normal again and grazing.

Sixty sheep were now taken and divided into two groups of thirty-five and twenty-five sheep; their ages varied from 2-tooth to adults, and consisted of ewes only. Faeces from twenty-five were taken from the rectum and all on examination showed *Gaigeria* eggs. As these sheep had not been selected from the flock but taken at random, except that all lambs were discarded, it appeared legitimate to conclude that if not all then a very high percentage of the adult sheep carried hookworm infection. In addition all the sheep showed numerous strongyle eggs, probably *H. contortus*, *Trichostrongylus sp.* and *Oesophagostomum columbianum* as revealed by the post-mortems on the sheep discussed above. The group containing thirty-five sheep was given each 5 c.c. C₂Cl₄ in an equal volume of a light mineral oil, while the other group received 10 c.c. C₂Cl₄ in an equal volume

of light mineral oil. Immediately prior to dosing with this drug each sheep received 10 c.c. of a 2 per cent. copper sulphate solution. These sheep were dosed during the late afternoon direct from the veld; during the evening it was noticed that some of the sheep in the group which had received the larger dose were slightly bloated and a trocar had to be used on one; the sheep in the other group showed no signs of bloating, although, after dosing, both groups became somewhat giddy and sluggish. Next morning, however, all the sheep were again normal. Two of the sheep from each group were now slaughtered, 13 hours after dosing, with the following results.

TABLE VIII.

Drug.	Amount.	Worms at post mortem.			
		Gaigeria.	H.c.	Tr.	O.C.
C ₂ Cl ₄	5 c.c.	Many	Numerous	Numerous	Numerous
"	10 c.c.	"	"	"	"
"	"	—	—	—	"

The two sheep which had received the larger dose showed no hookworms in the small intestine. Among the faeces of the large intestine, however, numerous dead hookworms were found. The other two sheep showed no dead hookworms in their faeces.

The above experiment confirmed the results obtained at the laboratory (vide Table V), namely that a dose of 5 c.c. C₂Cl₄ was not sufficient to remove the hookworms, whereas that of 10 c.c. was very effective. A large number of sheep was used in this experiment in order to see whether bloating was generally associated with the administration of the drug, and if so whether it had to be considered a serious reason against its use. From the above result the writers are inclined to the view that sheep after adequate dosing should be carefully watched during the ensuing few hours and any sheep showing marked bloating should be relieved: notwithstanding this drawback, it is felt that, with careful handling, Tetrachlorethylene is a relatively safe drug for use, especially for the reason that since performing the above test, a large number of doses have been administered at the laboratory to sheep of different ages, in poor and good condition, starved and unstarved, watered and unwatered, with and without copper sulphate solution, but in not a single case was there any signs of bloating. (See also discussion on toxicity of Tetraethylene below.)

For the next test thirty-eight sheep similar to and from the same flock as those used in the foregoing test were used. These had slept overnight in a kraal adjacent to the sheep used in the foregoing test, and were dosed the next morning after drenching with Copper sulphate solution. Each sheep received 20 c.c. of a half-and-half mixture of C₂Cl₄ and light mineral oil; these sheep were then given free access to water, which they did not take, and placed in a nearby

paddock with plenty of green grass. For a few hours these sheep kept together and appeared very drowsy, but otherwise no serious after-effects were noted and by midday all were normal and grazing.

This experiment thus tended to show that sheep which had been kraaled overnight and dosed next morning, were less likely to bloat than those which had been dosed during the late afternoon direct from the veld.

The results so far obtained in the field with Mr. Theophilus' sheep may be summarised as follows:—

1. Six selected adult sheep and twenty-five adults taken haphazardly showed by faecal examination that they were badly infested with hookworms; the probability was thus that if not the whole adult flock then a very high percentage of them was infested.

2. All these sheep were also heavily infested with wire-, nodular- and trichostrongyle worms.

3. Of eight sheep which had received 10 c.c. C_2Cl_4 five had on post-mortem lost all their hookworms, wireworms and trichostrongyle worms. The remaining three still had all these worms. All eight still had numerous nodular worms.

4. Of two sheep which had received 5 c.c. C_2Cl_4 both on post-mortem still carried a heavy infection of all the abovenamed worms.

5. It appears safer to dose the sheep in the morning after having been kraaled overnight.

6. As the sheep which had become cured of hookworms had lost their entire worm burdens of the abomasum and small intestine, and as the non-cured appeared to have retained all these, it appears legitimate to conclude that the effectiveness of Tetrachlorethylene is almost entirely dependent on whether it reaches the abomasum direct or not. This conclusion is substantiated by the results obtained by the dosing of this drug without previous drenching with a Copper sulphate solution, where the effectiveness of this drug was shown to be very low.

As it had now been shown that Tetrachlorethylene was a very effective anthelmintic for the treatment of *Gaigeria* hookworm, provided it is dosed direct into the abomasum, it was decided to dose the whole flock, in batches of about two hundred every morning. Each adult sheep was to receive the full dose, i.e. 10 c.c. C_2Cl_4 + 10 c.c. neutral mineral oil. The dosing had to be repeated three times at intervals of 10 to 14 days, and 10 days after each dosing 50 or more faeces samples had to be taken from the rectum from a similar number of sheep taken from the flock at random; these faeces to be examined microscopically by the egg centrifuge flotation method for the presence or absence of hookworm eggs. It was considered that such a procedure would give a good indication of the progress of the cure or otherwise in the whole flock after one, two or three treatments. As twenty-five sheep taken at random had already shown to be all infected with hookworms and other strongyloid worms, it appeared legitimate to assume that prior to treatment all, or a very high percentage of the sheep, were infected with both hookworms and other strongyloids.

The dosing of the first ninety sheep on the first day was done personally and it was arranged that the owner should continue the first dosing and also carry out the required second and third treatments. The collection of the faeces was also done under the supervision of the owner, when 25 to 30 grams from each sheep were placed in a suitable tube in 10 per cent. formalin and sent to the laboratory for further examination. As the sheep from which the samples were taken were on each occasion taken at random it is possible that samples from the same sheep may have been collected on more than one occasion; the chances, however, are that on each occasion the sheep were different, or at least the greater number of them were.

The results after these treatments may be summarised as follows:

After 1st treatment of 54 samples taken:*—

- 23 (42·6 per cent.) were free of hookworm eggs (-).
- 15 (27·8 per cent.) were just positive for hookworm eggs (+).
- 3 (5·5 per cent.) had a light infection of hookworms (++).
- 9 (16·6 per cent.) had a fair infection of hookworms (+++).
- 4 (7·4 per cent.) had a good infection of hookworms (++++).

In addition all the sheep showed the presence of a considerable number of other nematode eggs (+++ to ++++).

After 2nd treatment of 50 samples taken from the whole flock:—

- 38 (76 per cent.) were free of hookworm eggs (-).
- 3 (6 per cent.) were just positive for hookworm eggs (+).
- 5 (10 per cent.) had a light infection of hookworms (++).
- 2 (4 per cent.) had a fair infection of hookworms (+++).
- 2 (4 per cent.) had a good infection of hookworms (++++).

With regard to other helminths these samples showed that:—

- 16 (32 per cent.) were free of all helminth eggs (-).
- 5 (10 per cent.) were just positive for helminth eggs (+).
- 16 (32 per cent.) showed a light helminth infection (++).
- 6 (12 per cent.) showed a fair helminth infection (+++).
- 7 (14 per cent.) showed a good helminth infection (++++).

After the 3rd treatment of 54 samples taken from the whole flock:—

- 54 (100 per cent.) were free of hookworm eggs (-).
- 45 (83·3 per cent.) were free of all helminth eggs (-).
- 4 (7·4 per cent.) were just positive for helminth eggs (+).
- 3 (5·5 per cent.) showed a light helminth infection (++).
- 2 (3·7 per cent.) still had a fair helminth infection (+++).

(*These samples were taken at random from the 90 sheep dosed personally.)

For practical purposes we can consider that the sheep which are just positive for hookworms (i.e. from 25 to 30 grams of whose faeces after sedimentation and centrifuging not more than 10 eggs are seen in 4 to 5 drops from the top of the centrifuge liquid) are also cured, as the few worms which they contain would not produce any clinical symptoms. We have then a percentage of sheep cured of hookworms of 70·4 per cent. after the first treatment, 82 after the second treatment and 100 after the third treatment; in addition we find a percentage cure from other helminths of 42 after the second treatment and 90·7 after the third treatment. If these figures, which statistically are highly significant, are indicative of the flock, then the efficacy of Tetrachlorethylene, dosed immediately after the administration of a 2 per cent. solution of Copper sulphate, is very satisfactory. (Fig.) In addition the margin of safety is very high, as some 1,400 doses were administered with only 7 deaths, all of which were accidental and due to the direct result of the passage of either the Copper sulphate solution or Tetrachlorethylene direct into the lungs. It is true that bloating was often a sequel of dosing, but when the sheep are dosed in the morning, after having been kraaled overnight, this reaction is not serious; besides it is doubtful whether this bloating can be considered to be a general sequel of Tetrachlorethylene administration; it is possible that some ingredient in the feed in this particular area is a predisposing cause, perhaps vermeerbossie (*Geigeria* spp.) which is very plentiful in this area.

In order that these highly significant results could be confirmed or not the treated sheep were personally examined 25 days after the last dosing and 50 faeces samples were again taken from different sheep. The improvement in the general condition of the treated ewes was striking; whereas prior to treatment they were all very thin, weak and anaemic, they were now in a very good and strong condition. Only three sheep in the whole flock were still poor, and one of these, the only one in the flock, showed clinical symptoms of verminosis (anaemia and swollen jaw). A post-mortem of this sheep showed about 50 hookworms and a good number of wireworms and nodular worms. Faeces from the other two showed no hookworm eggs on examination, but both still had a light infection with other worms. The 50 samples, taken at random, included faeces from these three sheep, and on examination the following results were obtained:—

- 47 (94 per cent.) free of hookworms (-).
- 1 (2 per cent.) just positive for hookworms (+).
- 2 (4 per cent.) fair infection with hookworms (+++).
- 27 (54 per cent.) free of all other worms (-).
- 15 (30 per cent.) had a very light infection with other worms (+).
- 4 (8 per cent.) had a light infection (++).
- 2 (4 per cent.) had a fair infection with other worms (+++).
- 2 (4 per cent.) had a good infection with other worms (++++)

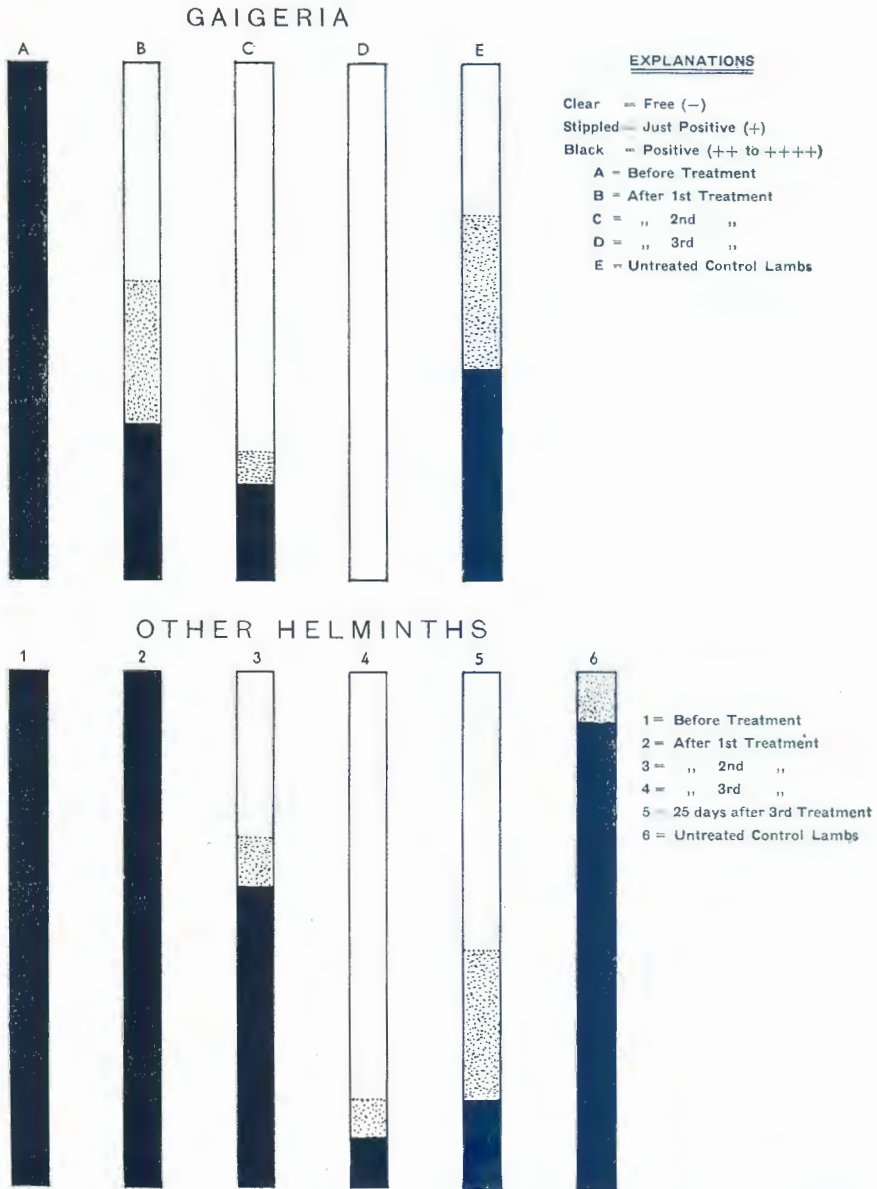


Fig. 1. Columns showing worm burden (*Gaigeria* and other Helminths) of flock before and after treatments with Tetrachlorethylene.

From these results it will be seen that whereas in the previous faeces samples taken 10 days after the third dosing all were free of hookworms in these latter, taken 25 days after the last dosing three showed hookworms eggs. This is not due to reinfection as it takes about 10 weeks for these hookworms to attain the egg laying stage in the host; the only explanation is that these sheep were infected prior to treatment and that as far as they were concerned the treatment was not successful, in that the drug was, at least in two of them, not swallowed direct into the abomasum. That the previous samples were all negative was simply a matter of chance, as the sheep from which they were taken were not selected. Taking the combined results of these two faecal examinations we still obtain very significant results, namely out of 104 samples there are 101 free of hookworms, and only 3 still infected, a percentage absolute cure of just over 97, which is very satisfactory.

That the loss of infection was not due to a natural loss of the hookworms is shown by about one hundred lambs which were running with the ewes; faecal examinations of ten of them showed that seven of them harboured a light hookworm infection at the time the last faeces samples were taken from the treated ewes. It may thus justly be concluded that these latter samples fully confirm the results of those taken previously; this view is also supported by post-mortem examinations done by the owner on four sheep which had died as a result of eating vermeerbossie (*Geigeria* sp.) a few days after the taking of the third batch of faeces samples; in these sheep there were no hookworms; neither were there any wireworms or trichostrongyles; three of the sheep were also free of nodular worms and one had very few.

As far as the other worms are concerned, the results obtained from an examination of this last lot of faeces is also very striking and supports the previous results. It is true that the infestation is now higher (54 per cent. free as against 83 per cent. free) but the intensity is not very much greater and can be very easily explained as due to reinfection; the period of 25 days since the last dosing is quite sufficient for wireworms and trichostrongyles to infect sheep and attain the egg-laying stage. The intensity of infestation with these worms is, however, very much lower than that in the one hundred lambs running with the ewes or in a flock of about three hundred and fifty lambs weaned some four months previously and running separately. As this infection was a mixed one, consisting of wireworms, trichostrongyles and nodular worms, and as the ewes harboured all these worms to a very marked extent prior to dosing, we are forced to the conclusion that their diminution is due to the dosing with Tetrachlorethylene.

That this drug has a toxic effect on hookworms, wireworms and trichostrongyloids is very easily appreciated, because, when dosed direct into the abomasum, the drug immediately or very soon comes into contact with these helminths in a very concentrated condition and its action is then very quick; in two sheep post-mortemed thirteen hours after dosing, no worms were found in the abomasum or small intestine, but numerous hookworms were present in the large intestine up to the anus; in ninety sheep, whose droppings

were examined four hours after dosing, a number were already passing dead hookworms. In both cases no search was made for wireworms or trichostrongyles. Its role, however, in the expulsion of the nodular worms is not so clear, and its effects on these worms became apparent only after the second and third dosings; it is possible that after the initial removal of the hookworms, wireworms and trichostrongyles, the balance between the nodular worms and their host became disturbed with the result that the worms were naturally got rid of. During the whole course of treatment no scouring of the sheep was noticed which would naturally tend to rid the sheep of a portion of its nodular worms.

While these experiments were being carried out, more detailed information became available with regard to stimulation of the oesophageal reflex and, in view of the fact that it appeared necessary to use 10 per cent. CuSO_4 for satisfactory stimulation in full-grown, poor-conditioned sheep, tests with 5 c.c. and 7.5 c.c. Tetrachlorethylene were again started on this basis. The following sheep were dosed with a mixture of equal quantities of the drugs and liquid paraffin, after a preliminary dose of 2.5 c.c. 10 per cent. CuSO_4 .

Dose of C_2Cl_4 .	Eggs at treatment.	Eggs 14 days after treatment.
5 c.c.	†	†
"	†	—
"	†	—
"	†	—
"	†††	—
"	††	††
"	††	†
"	††	—
"	††	—
"	††	—
7.5 c.c.	†††	††
"	††	†††
"	††	—
"	††	—

From these results it is seen that six out of nine sheep were freed of *Gaigeria* infestation by a single treatment with 5 c.c. of the drug, while two out of four were cleaned with 7.5 c.c. It therefore appears to be desirable to continue investigations in this direction in order to reduce the costs and risks of this treatment.

THE TOXICITY OF TETRACHLOROETHYLENE.

Information on the toxicity of this drug for sheep is urgently required. The experiments reported above and the experience of farmers have shown that the drug has two properties which are obviously undesirable. In the first place the drug is volatile and, even when given in mineral oil as a vehicle, sheep are very liable to cough and choke when the administration is not carried out with

great care. Secondly, absorption from the small intestine takes place very rapidly, since the sheep show signs of giddiness within a few minutes of treatment, even if they have swallowed the mixture satisfactorily, and the drug can be smelt in their breath.

The first difficulty cannot be obviated by administering the drug in a capsule, since it would then not be swallowed into the abomasum. Consequently attempts were made to find a better vehicle. Such a vehicle should comply with the following requisites: (1) It should bind the drug in such a way that no fumes escape at the moment of administration; (2) it should be inactive itself and should not be absorbed from the abomasum or intestine; (3) it should reduce absorption of the drug without binding the latter in such a way that it becomes less effective against the parasites. (It is known that some vehicles may enter into such a close combination, e.g. solution, with anthelmintics that the efficacy of the latter is reduced.) This third requisite is the most difficult to satisfy, but it would be worth while to investigate in this direction, since Tetrachlorethylene may become a satisfactory anthelmintic for eosophagostomes also if success could be achieved.

At first a series of heavy mineral oils, right up to vaseline, were tried and the heavy oils did to some extent satisfy points 1 and 2, but not 3, although efficacy was not reduced.

It was then found that Tetrachlorethylene (also Carbon tetrachloride) could easily be emulsified in the following way: To 25 c.c. of an aqueous solution of 7.7 gm. soft soap add successive small quantities of a mixture containing 37.5 c.c. Tetrachlorethylene and 37.5 c.c. liquid paraffin, while shaking vigorously. The emulsion obtained resembles thick cream and can be diluted with half a volume of soft water. It will be seen that 40 c.c. of the diluted emulsion contains 10 c.c. Tetrachlorethylene. This emulsion can be administered without the slightest risk of coughing or choking on account of fumes, because these are not given off. This satisfies point 1 completely. The emulsion is, however, broken by the acid in the abomasum and does therefore not have any further advantage. The sheep treated with 5 c.c. and 7.5 c.c. Tetrachlorethylene, reported on in the last test mentioned above, were dosed with this emulsion. Also in other cases it was found that the efficacy of the emulsion is quite satisfactory.

Continuing investigations in this direction it was found that emulsions could be made with gum arabic using various proportions of the drug and mineral oil, e.g. gum arabic 9 gm., water to 50 c.c. C_2Cl_4 50 c.c., liquid paraffin 25 c.c. Such an emulsion is not broken by acid and apparently passes through the abomasum. On reaching the small intestine it is, however, even more rapidly absorbed than a solution of the drug in mineral oil, causing more severe symptoms of giddiness. It has, however, the advantage that it can be mixed with solutions of magnesium sulphate or tannic acid, which would tend to decrease absorption. And indeed it was found that such combinations appeared to satisfy the requirements enumerated above, except that the efficacy against *Gaigeria* appeared to be greatly reduced.

It was further found that the addition of a small quantity of alkali (NaOH or NaHCO₃) to the gum arabic emulsion led to the rapid development of tympanitis in a fair proportion of the sheep, some dying as a result.

Returning to the soap emulsion it was found possible to add croton oil to the undiluted emulsion and then diluting as before. Sheep dosed with this preparation showed very little giddiness, the majority remaining quite normal after treatment. Doses of 1 and 2 c.c. croton oil were used. A treatment carried out on sheep infested with *Gaigeria*, using an emulsion as described above and containing in each dose 10 c.c. C₂Cl₄ and 1 c.c. croton oil gave the following result:—

Sheep.	Gaigeria and faeces passed in two days after treatment.	Eggs at treatment.	Eggs 14 days later.
1.....	3 S×N*	††	—
2.....	28 S×N	††††	—
3.....	21 S×N	†††	†
4.....	— S×N	††	—
5.....	20 N	††††	—
6.....	16 N	††	—

* S=soft. N=normal.

In tests on sheep infested with *Oesophagostomum columbianum* it seemed as if the efficacy was greater than that of an ordinary solution of Tetrachlorethylene and mineral oil.

The above are only preliminary records of the work done in connection with the undesirable effects of Tetrachlorethylene and the investigations will be continued.

SUMMARY.

1. Doses of 3, 4, 5, 6, 10 and 15 c.c. of pure Tetrachlorethylene per sheep are not efficient for the removal of the hookworm—*G. pachyscelis*.

2. Doses of 3, 4, 5 and 6 c.c. pure Carbon tetrachloride are not efficient for the removal of the hookworm—*G. pachyscelis*.

3. A dose of 8 c.c. of a half-and-half mixture of Tetrachlorethylene and Carbon tetrachloride is not efficient for the removal of the hookworm—*G. pachyscelis*.

4. Doses of 1, 3 and 5 c.c. of Stafford Allen's Extract of Pyrethrum M.225 had no effect in the removal of hookworm—*G. pachyscelis*.

ANTHELMINTHIC TESTS.

5. When dosed into the abomasum after administering 10 c.c. of a 2 per cent. Copper sulphate solution, 5 c.c. of Tetrachlorethylene in mineral oil was ineffective whilst 10 c.c. was very effective for removal of the hookworm—*G. pachycolis*.

After 2.5 c.c. of 10 per cent. Copper sulphate a dose of 5 c.c. was fairly effective.

6. n-Butyl chloride in 10 c.c. doses was only partially effective for the removal of the hookworm—*G. pachycolis*—when dosed after Copper sulphate solution.

7. In a flock of sheep badly infested with this hookworm and also with wireworms, trichostrongyles and nodular worms 10 c.c. pure Tetrachlorethylene in an equal volume of liquid paraffin administered three times immediately after drenching with 10 c.c. of 2 per cent. CuSO_4 solution at intervals of 10 to 14 days was very effective for the removal, directly or indirectly, of all these worms, giving a percentage cure of 97 for hookworms and 83 for all the other worms.

8. The undesirable effects of Tetrachlorethylene during administration, i.e. coughing and choking, can be overcome by using a soap emulsion of the drug.

9. Experiments carried out with a view to overcoming the rapid absorption of the drug from the alimentary tract and its sequelae, i.e. giddiness and reduced efficacy, are described.

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