

The Chemotherapy of Oesophagostomiasis in Sheep—II.

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THE experiments recorded in this paper include only tests which were based on administration of the drugs into the abomasum, i.e. in which the drugs were administered after a small quantity of copper sulphate solution—see paper by Mönnig and Quin in this issue. The work ran somewhat parallel to the investigations recorded in the latter paper and it will be seen that, as more knowledge was gained in regard to stimulation of the oesophageal reflex, the chemotherapeutic investigations profited by this knowledge.

Quite a number of the tests recorded below were carried out on farms in the vicinity of Pretoria, on account of the fact that it is as a rule difficult to produce artificial infestations with *Oesophagostomum columbianum* in a sufficiently large number of sheep and also because it was considered desirable to work under ordinary farming conditions. These circumstances did not always allow the tests to be carried out as exactly and the results to be followed up as far as one would wish, because the work entailed much transport and loss of time and in several cases the sheep were either sold or moved to another locality soon after the treatment. It was, however, usually endeavoured to attach faeces bags to the treated animals, to replace these daily for several days by fresh bags for the purpose of counting the worms passed after treatment, and to obtain faeces again about a fortnight after treatment for the purpose of making cultures from which the final results could be estimated. The original infestation of the sheep was estimated on the basis of reports made by the owners, post-mortems of one or more cases where possible and the clinical picture presented by the sheep selected out of the flocks for treatment. Although this method is not quite satisfactory, it was known that serious losses due to oesophagostomiasis were occurring on the farms selected and no other worms except *Haemonchus contortus* for which the sheep had as a rule been treated, play an important part in this area.

I wish to record my thanks to Mr. B. J. van der Vyver, Government Veterinary Officer of the Pretoria District, for his assistance in selecting the flocks on which these tests were carried out.

In the course of previous investigations (Mönnig, 1933), it had been found that certain relatively insoluble drugs appeared to produce the best results and such drugs were therefore now again tested in the first instance, the selection of the drugs being influenced to some extent by the previous results.

Test No. 1.

Out of a large flock of yearling merino lambs 30 moderately poor cases were selected and marked in six groups of five each. They were allowed to graze—on young succulent grass—and drank water immediately before treatment. Dosed 4 p.m. and the faeces bags removed 24 and 48 hours later. In each case the drug was administered immediately after 10 c.c. 1 per cent. copper sulphate solution.

Group 1—each 1 gm. *copper tartrate*. Passed 451 *Oes. col.** in 48 hours.

Group 2—each 0.95 gm. *arsenious sulphide*. One lamb passed 2 *Oes. col.*

Group 3—each 0.85 gm. *copper arsenate*. Passed 604 *Oes. col.*

Group 4—each 2.4 gm. *hexachlorethane*. No worms passed.

Group 5—each 0.6 gm. *barium arsenate*. Passed 94 *Oes. col.*

Group 6—each 0.4 gm. *mercurous sulphate*. Passed 11 *Oes. col.*

It is probable that in some cases worms were passed after the 48 hours. In groups 1 and 3 each of the lambs passed a number of worms each day but, as the lambs were not individually marked, individual records cannot be given. Some six weeks later the owner reported that the lambs of groups 1 and 3 had markedly improved but not the others. None of the lambs had appeared to suffer as a result of the treatment.

Test No. 2.

Sheep 1-2 years old, very poor and dull, grazing on green grass but had no water on the day of treatment. Stimulated with 10 c.c. 1 per cent. copper sulphate solution.

Group 1—8 sheep, each 0.5 gm. *copper arsenate*. Passed 48 *Oes. col.* in 3 days.

Group 2—8 sheep, each twice 0.5 gm. *copper arsenate* on two successive days. Passed 188 *Oes. col.* in 3 days from the first treatment.

Group 3—4 sheep, each 5 c.c. *tetrachlorethylene* in 10 c.c. liquid paraffin. Passed 93 *Oes. col.* in 3 days.

Faeces cultures made a fortnight later showed that the sheep of group 2 were free of nodular worms, while those of the other two groups were still infested. Again the sheep had not been individually numbered, hence it cannot be stated whether all or only some in each group passed worms.

In the light of knowledge which was gained later it appears probable that the copper sulphate solution was too weak to produce a satisfactory stimulus in some cases and also that the single dose of 0.5 gm. copper arsenate was insufficient. The test had been carried out mainly to ascertain whether such a dose would be sufficient.

* *Oes. col.* = *Oesophagostimum columbianum*.

Test No. 3.

At Onderstepoort three poor, weak, adult sheep were dosed with 0.85 gm. *copper arsenate* each after 10 c.c. 1 per cent. copper sulphate. The following numbers of *Oes. col.* were passed:—

	1st day after treatment.	2nd day.	3rd day.	4th day.	5th day.	Total.
Merino 41482.....	7	5	48	30	0	90
Merino 41483.....	21	7	38	18	0	84
Persian 41466.....	0	1	0	0	0	1

Subsequent examination showed that the two merinos were clean but the persian still infested. The latter had probably swallowed the drug into the rumen.

Test No. 4.

Merinos about 2-3 years old, in poor condition, grazing on green grass but had no water on day of treatment. These sheep were at the time not badly infested as they had been given enema treatments on several occasions. Stimulated with 10 c.c. 1 per cent. copper sulphate.

Group 1—5 sheep, each 0.85 gm. *copper arsenate*. Three passed no worms, one 9 and one 190 *Oes. col.* in three days.

Group 2—4 sheep, each 1 gm. *copper tartrate*. Passed 11, 24, 30 and 117 *Oes. col.* respectively in 3 days.

Group 3—5 sheep, each 1 gm. *cryolite* (mixture of fluorides). Passed 11, 3, 2, 3, 3 *Oes. col.* respectively in 3 days.

Group 4—5 sheep, each 5 c.c. *tetrachlorethylene* in 15 c.c. skimmed milk. Passed 0, 0, 1, 1, 3 *Oes. col.* respectively in 3 days.

The sheep were not re-examined later.

Test No. 5.

Merinos, 2-tooth, moderate to poor condition but well grown. Fed on dry hay and not kept from water. Stimulated with 10 c.c. 1 per cent. copper sulphate.

Group 1—2 sheep, each 5 c.c. *tetrachlorethylene* in 15 c.c. liquid paraffin. Only one passed one *Oes. col.* Later both still infested.

Group 2—2 sheep, each 0.42 gm. *mercurochrome*. Only one passed two *Oes. col.* Later both infested.

Group 3—2 sheep, each 1 gm. of a relatively insoluble proprietary drug stated to possess anthelmintic properties. No worms passed.

Test No. 6.

Merinos and crossbred sheep in poor condition, 1-2 years old. Running on green pasture, not kept from water. Stimulated with 10 c.c. 2 per cent. copper sulphate.

Group 1—5 sheep, each twice 0.95 gm. *arsenious sulphide* (on two successive days). Passed no worms. Later still infested.

Group 2—5 sheep, each twice 1.1 gm. *copper carbonate*. Only one passed one *Oes. col.* Later still infested.

Group 3—5 sheep, each twice 1.75 gm. *bismuth subnitrate*. Passed no worms. Later still infested.

Group 4—5 sheep, each 1 gm. *lead tartrate*. Passed 23, 1, 0, 0, 0 *Oes. col.* respectively. Later still infested.

Group 5—5 sheep, each 1 gm. *lead arsenate*. Passed 5, 9, 10, 15, 0 *Oes. col.* respectively. Later still infested.

Group 6—5 sheep, each 1 gm. *bismuth subgallate* ("Derma-tol"). Passed no worms. Later still infested.

In the above tests copper arsenate and copper tartrate were the two outstanding drugs which gave the most satisfactory results and further tests with them were subsequently made, as will be reported below.

TESTS WITH TETRACHLORETHYLENE.

In other anthelmintic work indications had been obtained to the effect that tetrachlorethylene may sometimes be fairly effective against the nodular worm and further tests with this drug were made at Onderstepoort in order to obtain more information.

Tests with this drug on 11 sheep have been mentioned above and the results were not promising. In some cases the 5 c.c. doses were quite large enough considering the size and condition of the sheep, in other cases they could have been larger. Also, it is possible that the copper sulphate solution used was too weak.

In the further tests 5 per cent. copper sulphate was used as stimulant and the doses of tetrachlorethylene was usually 5 c.c. for lambs of 6-12 months and 10 c.c. for sheep over 12 months. The drug was mixed with mineral oils of various specific gravities and viscosities and other vehicles and adjuvants were tested.

Since this work is still in progress it will be reported upon in a later article, but at this stage it may be mentioned that, although it may be possible to arrive at an effective formula, a simple combination of tetrachlorethylene and a mineral oil does not appear to be satisfactory. The doses required to clean sheep of nodular worms after several treatments are on the border of the limit of safety and further study of the conditions under which tetrachlorethylene is toxic to sheep is urgently required. Moreover, the cost of repeated doses as required for a complete cure are relatively high and would be prohibitive under certain conditions.

FURTHER TESTS WITH COPPER ARSENATE AND COPPER TARTRATE.

Test No. 7.

A flock of full-grown merinos in poor condition and rather dull, grazing on slightly dry pasture, kept from water for 24 hours before and 18 hours after treatment.

Group 1—70 sheep, stimulated with 10 c.c. 1 per cent. copper sulphate, dosed each 0.6 gm. *copper arsenate*. Five of the poorest sheep of this group passed 73, 29, 29, 0, 0 *Oes. col.* in 48 hours. One very poor sheep in this group (not of the last five used for worm counts) killed three days after treatment had about 200 *Oes. col.* still alive.

Group 2—Of 5 poor sheep, stimulated with 10 c.c. 2 per cent. copper sulphate and dosed each 0.6 gm. *copper arsenate* only one passed 13 and another 1 *Oes. col.* in 48 hours.

Group 3—90 sheep, stimulated with 10 c.c. 1 per cent. copper sulphate, dosed each 1 gm. *copper tartrate*. Five of the poorest were selected for worm counts; only one passed 10 *Oes. col.*

Group 4—Of 5 poor sheep, stimulated with 10 c.c. 2 per cent. copper sulphate and dosed each 1 gm. *copper tartrate* only one passed 1 *Oes. col.*

The doses of the drugs used were apparently too small for these full-grown sheep, and, according to later experiences, the stimulant was also too weak.

TOXICITY TESTS.

Since nothing was known with regard to the toxicity of these two drugs it was considered advisable first to gain some knowledge on this point.

Test No. 8.

A mixed lot of persian and cross-bred sheep ranging in age from about 5 months to 2 years, all except some of the older ones in moderate or poor condition. Grazing was green and the sheep were allowed to drink immediately before treatment. Stimulant 10 c.c. 2 per cent. copper sulphate.

Group 1—40 sheep of various ages dosed each 1 gm. copper arsenate. Two days after treatment a lamb of 4-5 months died with lesions of arsenical poisoning and another of the same age was very ill but recovered.

Group 2—40 sheep of various ages dosed each 1 gm. copper tartrate. These sheep remained quite normal.

The owner later reported that these sheep had improved appreciably in condition after treatment.

CHEMOTHERAPY OF OESOPHAGOSTOMIASIS IN SHEEP.

Test No. 9.

Merinos in moderate to poor condition but well grown, age 2-tooth.

Feeding on dry hay, not kept from water. Stimulant 10 c.c. 1 per cent. copper sulphate.

Group 1—7 sheep, each twice (on two successive days) 1.25 gm. *copper arsenate*. Four died of arsenical poisoning.

Group 2—2 sheep, each twice 2 gm. *copper arsenate*. Both died of arsenical poisoning.

Group 3—4 sheep, each one dose 4 gm. *copper arsenate*. Two died of arsenical poisoning.

Group 4—5 sheep, each twice 1.65 gm. *copper tartrate*. No adverse effects.

Group 5—6 sheep, each twice 2 gm. *copper tartrate*. One died of copper poisoning.

Group 6—2 sheep, each twice 2.5 gm. *copper tartrate*. One died of copper poisoning.

Group 7—3 sheep, each one dose 4 gm. *copper tartrate*. No adverse effects.

Group 8—2 sheep, each one dose 5 gm. *copper tartrate*. No adverse effects.

Group 9—6 sheep, each one dose 6 gm. *copper tartrate*. 3 Died of copper poisoning, two others showed symptoms—marked icterus and methaemoglobinuria—but recovered.

Most of these sheep had been infested with nodular worms before treatment but were clean thereafter.

According to the results of the above tests it would appear that a double dose of 1 gm. copper arsenate or a double dose of 1.5 gm. copper tartrate is within the limits of safety for sheep over 18 months old. Since an overdose of the former leads to arsenical poisoning and an overdose of the latter to copper poisoning, a combination of the two drugs, which would allow cutting down the quantity of each, was now considered, particularly because it was though possible that the two drugs acted on the parasites in different ways.

Test No. 10.

110 Cross-bred sheep, various ages from about 5 months to 2 years. Grazing on green pasture and allowed to drink immediately before treatment. Stimulated with 10 c.c. 2 per cent. copper sulphate. Dosed with copper arsenate 1 part and copper tartrate 2 parts, giving on each of two successive days to lambs up to six months 0.72 gm. (0.24+0.48 gm.), 6-18 months 1.1 gm. (0.37+0.73 gm.) and sheep over 18 months 1.45 gm. (0.48+0.97 gm.). The owner reported later that four days after the second dose a small, weak lamb had died but the other sheep showed no ill effects and improved much after the treatment.

TESTS WITH MIXTURES.

Test No. 11.

Group 1—5 cross-bred sheep in fairly poor condition, 1-2 years old, grazing on green pasture and not kept from water. Stimulated with 10 c.c. 2 per cent. copper sulphate. Dosed on each of two successive days with 1.1 gm. of mixture as used in test No. 10. Passed 77, 37, 18, 0, 0 *Oes. col.*

Group 2—4 cross-bred sheep similar to group 1 and similar treatment but dosed twice with 1.25 gm. of a mixture copper arsenate: copper tartrate=2:3 (i.e. 0.5 gm. + 0.75 gm.). Passed 139, 99, 32, 1 *Oes. col.*

In both groups no ill effects were noticed and faeces cultures a fortnight later showed that the sheep were all free of nodular worms.

Group 3—About 100 cross-bred sheep of the same flock, treated like the other two groups but dosed twice with 1.4 gm. of a mixture of copper arsenate: copper tartrate=2:5 (i.e. 0.4 gm. + 1.0 gm.). No faeces were collected but the owner reported later that the flock had completely recovered.

In this connection it should be stated that, when faeces were collected from groups 1 and 2 for cultures a fortnight after treatment the whole flock was treated for infestation with *Oestrus ovis* which appeared to affect a number of the sheep, and which may have accounted for the poor condition, because the majority of the sheep had relatively few nodular worms and some none at all, at the time of treatment. In several instances it has been noticed that the combination of nodular worm and *Oestrus ovis* is very severe on sheep. The nasal maggots produce an irritation which keeps the sheep from feeding properly and an insufficiency of food has very serious effects on sheep infested with nodular worms.

Test No. 12.

A flock of 160 full-mouth merinos in very poor condition and rather dull. Grazing on pasture which, though still green, was becoming coarse. Kept from water from 24 hours before first dose until 12 hours after second dose. Stimulant 10 c.c. 2 per cent. copper sulphate. Dosed each twice with 0.5 gm. copper arsenate and 1.25 gm. copper tartrate (i.e. 2:5). Twenty-four of the poorest sheep were selected for collection of faeces and all except four passed nodular worms. They had not been individually numbered, but the total of worms passed was 469, giving an average of 23.5 for the 20 sheep.

A week after treatment the three poorest sheep of the flock were killed; they still harboured roughly 50, 150 and 200 nodular worms.

The day after the second dose two sheep died and the next day another one, apparently of arsenical poisoning. The whole flock appeared to be affected by the treatment and did not improve much later on.

CHEMOTHERAPY OF OESOPHAGOSTOMIASIS IN SHEEP.

This result led to further toxicity tests:—

Test No. 13.

Sheep at Onderstepoort, full grown, 4-6 tooth, in medium to poor condition. Fed dry hay and crushed maize. Stimulant 10 c.c. 2 per cent. copper sulphate.

Group 1—50 sheep. Not kept from water but did not drink within a few hours of treatment. Dosed twice with 0·8 gm. copper arsenate and 1·2 gm. copper tartrate. Two sheep died of arsenical poisoning after the first dose. Another five died of arsenical poisoning after two doses.

Group 2—20 sheep, kept from water on the days of treatment, dosed at 2 p.m. Each received twice 0·6 gm. copper arsenate and 1·5 gm. copper tartrate. Not affected by treatment, no deaths.

Group 3—18 sheep, treated like group 2 but dosed each twice 0·6 gm. copper arsenate and 1·2 gm. copper tartrate. One sheep died a day after the second dose but showed no lesions and the liver contained only traces of arsenic.

Group 4—2 lambs about 4 months old, treated like group 2 but dosed each twice 0·4 gm. copper arsenate and 0·8 gm. copper tartrate. No ill effects.

Group 5—20 sheep, kept from water from 24 hours before first treatment until 6 hours after second treatment. Dosed each twice with 0·6 gm. copper arsenate and 1·5 gm. copper tartrate. One sheep died of arsenical poisoning a day after the second dose and another four days later. The other sheep did not appear to be badly affected but some were rather dull for a few days.

Group 6—20 sheep, treated like group 5 but dosed each twice with 0·7 gm. copper arsenate and 1·75 gm. copper tartrate. Two sheep died after the first dose; one, very poor and weak, died 8 hours after treatment and had all of the remedy still in the abomasum and there was a slight hyperaemia of the mucosa. The other sheep, in fair condition, died 24 hours after treatment, having been prostrated for several hours, and very little of the remedy had yet left the abomasum. There was an acute abomasitis and the liver showed a sufficient quantity of arsenic (0·6 mg. As_2O_3 per 100 gm.) to confirm the diagnosis of arsenical poisoning. Three more sheep died of arsenical poisoning two days after the second dose. The rest all appeared to be slightly affected for a few days.

Group 7—20 sheep, treated like group 5 but dosed each twice with 0·8 gm. copper arsenate and 2 gm. copper tartrate. Three sheep died of arsenical poisoning, one, three and six days respectively after the second dose. The others also were slightly affected for a few days.

In considering the results of tests Nos. 12 and 13 two points have to be taken separately.

Firstly the question whether the drugs were swallowed into the abomasum in all cases. From information which was obtained at a later stage and which is discussed in another paper (see article by Mönnig and Quin in this issue) it appears very probable that this was not the case. It was later found that full-grown sheep, and particularly if they are in poor condition, are not well stimulated by 2 per cent. copper sulphate. In addition there is the factor of fluidity of the ruminal contents—if the contents are fairly fluid the drug passes to the abomasum more frequently than when the contents are on the dry side, and the latter was probably the case with many of these sheep, especially groups 2-7 which were receiving dry food and were kept from water. If the drug falls into the rumen it is practically lost as far as the nodular worm is concerned, because the small quantities which would pass through over a prolonged period would probably not reach the worms, since the drug would become dissolved in the abomasum and be absorbed. Since the absorption would be slow and spread over a considerable period of time, the danger to the sheep would be relatively small. In test No. 12 probably one or both of the two doses were in many cases swallowed into the rumen, hence the low efficacy. The fact that the three poorest sheep were eventually killed for examination is also significant because these were the worst cases for stimulation and would therefore show the least successful result.

Secondly there is the question what happens when the remedy is swallowed into the abomasum. Both the drugs are relatively insoluble in water but more soluble in an acid medium. The following degrees of solubility were determined by Mr. P. M. Bekker of the chemistry section. Solubility at $\pm 42^{\circ}\text{C}$.:—

	In 0.25% HCl.	In 1% HCl.
Copper arsenate.....	0.44%	1.80%
Copper tartrate.....	0.64%	1.44%

Information on the acidity of the abomasal contents of sheep appears to be scanty but it seems that the degree of acidity may vary from about neutral to 0.1 per cent. or even more (HCl) and would be highest in the case of sheep in active process of digestion. It also seems reasonable to conclude that when concentrates are fed and water is withheld there would be a high degree of acidity.

Further, the length of time the drugs remain in the abomasum would be important, since a long stay would afford greater opportunity for the drugs to become dissolved. In group 6 the drugs remained in the abomasum for 8 hours in one case and about 24 hours in another. Although these periods are abnormally long, a similar state of affairs may have obtained in some of the other sheep.

One may therefore conclude that, if the degree of acidity in the abomasum is high the drugs will become dissolved to a fair extent. The dissolved drugs would then be readily absorbed and may lead to poisoning of the sheep and furthermore they could not be effective against the nodular worms in the colon. The longer the drugs remain

in the abomasum the greater becomes this risk. Unfortunately we have no definite knowledge to-day in regard to the physiology of the abomasum and the factors which control the passage of ingesta through the pylorus on which one could base attempts to accelerate the passage of drugs through the abomasum. While starvation is obviously contraindicated, it would seem that the most favourable condition to be aimed at under the circumstances is that of normal, active digestion in which the ingesta pass through the abomasum and intestine at a reasonable speed. Water should not be withheld and fresh, green grass appears to have a favourable influence.

If one has to deal with a reasonably short stay of the drugs in the abomasum, it may be possible to neutralise the acid for that period and this was therefore the next step contemplated. Both the drugs are relatively soluble in an alkaline medium, as can be readily shown in a test-tube. Since the amount of acid in the abomasum varies within wide limits, a neutralising agent given in sufficient quantity to cope with a high degree of acidity would often be in excess if acidity is low and should then not produce a sufficient degree of alkalinity to dissolve the drugs. The neutralising agent has therefore to be an alkaline substance which does not ionise to any marked extent. These requirements appear to be satisfactorily fulfilled by slaked lime (calcium hydroxide). Tests in the laboratory showed that when the drugs were added to dilute hydrochloric acid, the addition to the drugs of slaked lime in excess of the acid produced an almost neutral medium with no appreciable solution of the drugs, while sodium bicarbonate instead of slaked lime produced an alkaline medium and a fair proportion of the drugs became dissolved. Subsequent tests on sheep also showed that sodium bicarbonate did not improve matters while slaked lime brought about a definite improvement.

Test No. 14.

The following mixtures were tested:—

	Dose* for sheep—		
	3-6 months.	Over 6 to 18 months.	Over 18 months.
<i>Mixture I.</i>	Gramme.	Gramme.	Gramme.
Copper arsenate, 2 parts.....	0.3	0.4	0.5
Copper tartrate, 5 parts.....	0.75	1.0	1.25
	1.05	1.4	1.75
<i>Mixture II.</i>			
Copper arsenate, 2 parts.....	0.3	0.4	0.5
Sodium bicarbonate, 3 parts.....	0.45	0.6	0.75
Copper tartrate, 5 parts.....	0.75	1.0	1.25
	1.5	2.0	2.5
<i>Mixture III.</i>			
Copper arsenate, 2 parts.....	0.3	0.4	0.5
Calcium hydroxide, 3 parts.....	0.45	0.6	0.75
Copper tartrate, 5 parts.....	0.75	1.0	1.25
	1.5	2.0	2.5

* Single dose, to be repeated the following day.

A flock of 523 sheep running on green grass, all well over 18 months old, was treated as follows:—

The poorest sheep were selected, some being in a very bad condition. This lot of 146 was divided into four groups:—

Group 1—25 sheep, 24 hours without water, drank immediately before treatment. Mixture I.

Group 2—48 sheep, 24 hours without water, drank immediately before treatment. Mixture III.

Group 3—25 sheep, 24 hours without water, drank immediately after treatment. Mixture I.

Group 4—48 sheep, 24 hours without water, drank immediately after treatment. Mixture III.

The rest of the flock, in moderate to good condition, was allowed to graze and drink and some drank immediately before treatment.

Group 5—50 sheep, mixture I.

Group 6—100 sheep, mixture II.

Group 7—227 sheep, mixture III.

All the sheep were stimulated with 10 c.c. 2 per cent. copper sulphate and given a double dose, i.e. on two successive days.

Groups 1-4 were kept in a suitable enclosure overnight after both treatments and the droppings showed that numerous nodular worms had been passed. The shepherd also reported that worms were passed during the daytime up to three days after the second treatment. Groups 5-7 also showed nodular worms in many lots of faeces found in their pasture. The sheep of groups 2, 4 and 7 which had received the mixture containing slaked lime showed no ill effects and definitely looked more lively than many of the other sheep did shortly after treatment.

A day after the second dose one sheep each of groups 1, 3 and 6 died of arsenical poisoning and during the following two days another two of group 6 died, thus showing apparently that the sodium bicarbonate was not satisfactory.

From the number of worms passed by groups 1-4 it was obvious that a considerable proportion of the infestation had been removed from the flock, but whether the treatment had been effective in all cases, especially the very poor sheep, remained to be seen. The five poorest sheep were therefore selected from each of these four groups

(1-4) and slaughtered. They harboured the following numbers of nodular worms (given in round numbers in the positive cases with a fair or heavy infestation):—

Group 1—0, 0, 11, 50, 200.

Group 2—0, 0, 80, 150, 200.

Group 3—5, 20, 26, 120, 120.

Group 4—0, 80, 100, 200, 200.

This result would seem to indicate that, as has been mentioned before and was later confirmed, sheep in very poor condition are bad subjects for stimulation of the oesophageal reflex and that the efficiency of treatment depends not only on the drugs used but to an equal degree on the route of passage through the stomach.

Another flock of 300 yearling lambs in moderately poor condition were treated, again using 10 c.c. 2 per cent. copper sulphate as stimulant and giving a double dose of mixture III. These lambs grazed on green grass and were allowed water freely. Many of them drank shortly after being given the first dose and also shortly before the second dose. During the night after the first dose many worms were passed. After having received the second dose the sheep were driven slowly to another farm and the owner reported later that no ill effects had been observed and the lambs had improved markedly.

Test No. 15.

A toxicity test was now made with mixture III as used in test 14. The sheep were in good condition and were fed dry hay and crushed maize. They were kept from water for 24 hours before each treatment with the intention of getting them to drink immediately before dosing, but the weather was cold and on both days only a few of the sheep drank a little. Again 10 c.c. 2 per cent. copper sulphate was used as stimulant.

Group 1—7 sheep, each twice 3 gm. mixture III (copper arsenate 0·6, lime 0·9, copper tartrate 1·5 gm.).

Group 2—7 sheep, each twice 4 gm. mixture III (0·8, 1·2, 2·0 gm.).

Group 3—6 sheep each twice 5 gm. mixture III (1·0, 1·5, 2·5 gm.).

No ill effects were observed in any of these sheep.

The day after the second dose one sheep of each group was selected which had swallowed one or both doses into the abomasum, as far as this could be ascertained from the appearance of the faeces. These three sheep were killed and carefully examined with the following results:—

Group 1—normal except for a very slight hyperaemia of the duodenum, which would be of practically no consequence. Liver shows a trace of arsenic.

Group 2—normal. Liver shows a trace of arsenic.

Group 3—a very slight hyperaemia of the abomasal mucosa and the anterior 15 cm. of the duodenum; of no practical importance. Liver shows a trace of arsenic.

Test No. 16.

A critical test with mixture III, giving the same doses as in test 14, was now made on some very poor sheep, using 10 c.c. 5 per cent. copper sulphate as stimulant. In the experiments on deglutition it had meanwhile been found that a stronger bluestone solution gave a better stimulus in such poor sheep. Some of the sheep died of poverty shortly after treatment while the others were killed in deglutition tests and in every case the intestines were examined for the presence of nodular worms.

Age of sheep.	Nodular worms passed.	Worms remaining.	Approximate efficacy.
4-tooth.....	6	about 30	0
	171	30	0·5
	82	0	1
6-tooth.....	212	0	1
	48	about 20	0·5
9 months.....	177	0	1
	1	0	1
	2	2	0·5
	10	0	1
	26	0	1
	157	0	1
	892	82	8·5/11=77%

This result was not yet quite satisfactory, especially with regard to the older sheep. Some may have swallowed only one of the two doses into the abomasum. It was later found that 10 per cent. copper sulphate produced better results in deglutition tests. Further tests were made on farms with 5 per cent. copper sulphate as stimulant and using the same doses of mixture III as in test 14. In all cases the results were satisfactory, i.e. many worms were passed and the sheep improved in condition.

In one case the first opportunity arrived of treating young lambs of 3-4 months. They were in very poor condition, weak and rather small for their age. The lambs were separated from the ewes for four hours before up to three hours after treatment. A double dose of mixture III was given (each dose copper arsenate 0·3 gm., lime 0·45 gm., copper tartrate 0·75 gm.). Of 80 lambs so treated 30 died within a fortnight. Unfortunately the owner made no observations with regard to the cause of death and only reported the deaths a few weeks later. However, the proportion of deaths was higher than in the untreated controls. The owner then treated another 50 lambs with a single dose. These passed many worms and one death occurred about 10 days after treatment, probably on account of weakness.

CHEMOTHERAPY OF OESOPHAGOSTOMIASIS IN SHEEP.

Since there appeared to be a certain degree of danger in using the doses previously arrived at, it was decided to reduce the doses for young sheep and the following was now decided on:—

	Dose for—		
	3-6 months.	Over 6 to 18 months.	Over 18 months.
	Gramme.	Gramme.	Gramme.
Copper arsenate.....2	0.2	0.36	0.5
Calcium hydroxide.....3	0.3	0.54	0.75
Copper tartrate.....5	0.5	0.9	1.25
Dose No.....	1: 1.0	2: 1.8	3: 2.5

Sheep in very poor condition should receive the next smaller dose on the second day, very poor lambs only one dose.

According to these measures about 2,000 sheep of various ages, including young lambs, were treated on seven farms in different parts of the Union. The treatment was carried out by farmers with the assistance of Government Veterinary Officers. For stimulation 5 per cent. copper sulphate was used. No deaths occurred as the result of treatment. In some cases faeces were collected and the droppings of other sheep were examined where the animals had slept after treatment. The impression was obtained that a large proportion of the infestation was eliminated. Incidentally it was noted that many *Haemonchus contortus* and *Moniezia* were also passed and heads of the tapeworms were recovered from the faeces in some cases.

Meanwhile it had been determined that a 10 per cent. copper sulphate solution produced satisfactory stimulation even in very poor conditioned, full-grown sheep and that 2.5 c.c. was sufficient for this purpose. It was therefore decided to test the efficacy of the treatment again with the stronger stimulant.

Test No. 17.

Ten 2-tooth sheep in very poor condition and rather dull, feeding on dry hay and crushed maize, not removed from water. Stimulant 2.5 c.c. 10 per cent. copper sulphate. First day dose No. 2, second day dose No. 1.

According to faeces cultures made seven days before treatment all these sheep had nodular worms, though not severe infestations. Two had *Haemonchus contortus*. They passed respectively 16, 9, 52, 3, 17, 16, 20, 32, 23 and 203 (total 391) nodular worms. Three died six to fourteen days after treatment of poverty and had no worms left. Faeces cultures of the other sheep showed no residual infestation with nodular worms or *Haemonchus* except one culture in which a single oesophagostome larva was found. Some of these sheep were later again examined and treated but appeared to be free of nodular worms.

Unfortunately no older sheep in poor condition with nodular worm infestation were available for these tests. Such sheep are important as reservoirs of infection and therefore require to be cleaned. As a rule, however, it is the young sheep up to 2-tooth that suffer and for which a remedy is mainly required. Judging from the results obtained in deglutition tests on old, poor conditioned sheep, it is very probable that two or three treatments would clean such cases, while many would be cleaned already by one treatment.

Test No. 18.

Although test No. 17 had shown that one treatment may suffice for relatively young sheep, repeated treatments would probably be given in practice on farms and it was therefore considered desirable to test the safety of repeated treatments, at short intervals.

A month after test No. 17 two of the sheep used in that experiment and another four of which one was infested, were treated giving dose No. 2 each time. All these sheep were in very poor condition, the state which is reached by some sheep with nodular worm infestation when, even if the parasites are removed, the animals hang on and either die after weeks or recover very gradually. The one infested sheep passed 31 nodular worms and none of the animals showed any adverse effects.

A week later the treatment was repeated, again giving full doses. No worms were passed and no adverse effects were noted.

Another week later the same treatment was repeated and two new infested sheep, also in poor condition, were included. These latter two passed all their nodular worms. Again no ill effects were noted.

It appears therefore that treatment can safely be repeated at short intervals, although in practice intervals as short as seven days would rarely be contemplated.

DISCUSSION.

If the results are considered together with the work done on stimulation of the oesophageal reflex, which ran parallel, it becomes quite clear that the question of deglutition is an important part of the treatment against oesophagostomiasis and that it will be equally important in the chemotherapy of other gastro-intestinal worm diseases of ruminants.

The mixture of copper arsenate, calcium hydroxide and copper tartrate appears to fulfil the requirements of an effective remedy against nodular worm in sheep. If the remedy does not have the desired effect, the evidence appears to show that deglutition into the abomasum was not satisfactory.

The remedy appears to stimulate secretion of mucus in the colon, so that in many cases the faeces become soft or mixed with a fair amount of mucus and sometimes a sheep may even pass a mass of mucus. In a few cases it has been observed that live nodular worms

were passed with such mucus, as may be expected. The remedy does, however, not act by virtue of this property, since in many of the cases on which careful observations were made the worms were passed in normal or slightly pasty faeces.

It may be necessary to explain why the idea of double dosing was adopted. The reasons will be given in order of their estimated importance:—

1. In the case of a parasite with feeding habits such as those of *Oesophagostomum columbianum* and a drug which probably acts as an internal poison it appears desirable to have a long period during which action of the drug on the parasite is possible.
2. Since the oesophageal reflex cannot yet be stimulated in 100 per cent. of cases, but in well over 50 per cent., and since the efficacy of one of the doses is very probably more than half that of the two doses, some sheep which swallow one dose into the rumen may swallow the other into the abomasum and the total result in a flock will be better than if a single large dose is given.
3. The total quantity of the drugs administered can be greater and each dose smaller than one single dose.

It is not claimed that a completely satisfactory remedy has been found for oesophagostomiasis in sheep. Further tests on a large scale will have to show what degree of efficacy can be obtained under different conditions and whether certain yet unknown factors may not introduce an element of danger. Moreover, further possible improvements with respect to stimulation of the oesophageal reflex may improve the results. It is, however, expected that a few treatments at the correct time of the year will stop the disease and lead to satisfactory control of the parasite, particularly if treatment is carried out with a view to removing the source of infection at a suitable season.

The Department of Agriculture has decided to issue to farmers about half a million double doses of this remedy for the purpose of a test. The directions for use issued with the remedy are given below and from these it can be seen in which way it is intended that the remedy should be used in the treatment and control of the disease. It might be added here that the disease is mainly important in areas with a summer rainfall (October to March) and a relatively dry winter.

With regard to costs it may be stated that, if the remedy should prove satisfactory and is later issued on a large scale, it is expected that the costs should not be higher than about 1s. per 100 double adult doses.

I would like to express my thanks to Drs. Malan and Graf and other members of the Chemistry Section for making the copper arsenate and copper tartrate used in these tests and for assistance in various other ways.

SUMMARY.

1. Chemotherapy for oesophagostomiasis must be based on the administration of the remedy into the abomasum. At first a 1 per cent. copper sulphate solution was used for stimulation of the oesophageal reflex, then higher concentrations until finally satisfactory results were obtained with 2.5 c.c. of a 10 per cent. solution.

2. Preliminary tests were made with 15 different chemicals which had either previously given indications that they may be effective, or appeared, according to their chemical and physical properties, to be suitable. Of these copper arsenate and copper tartrate gave rather outstanding results.

3. Both these drugs were found to be dangerous in doses which would be large enough to effect a cure. An overdose of copper arsenate leads to arsenical poisoning while an overdose of copper tartrate leads to copper poisoning.

4. A mixture of the two drugs produced variable results which were found to be connected with the variable amount of acid in the abomasum. Since the drugs are relatively soluble in acid and alkaline media, high stomach acidity would cause solution and absorption of the drugs with consequent poisoning of the sheep and little or no effect against the parasites. Calcium hydroxide was found to be a suitable corrective and was incorporated in the mixture. Effective doses of this mixture were found to possess a suitable degree of safety, even if treatment is repeated at relatively short intervals.

5. Taking into account all ages and conditions of sheep, the efficacy of one treatment, i.e. a dose on each of two successive days, is expected to reach at least 75 per cent. If only young sheep under 2 years of age are treated the efficacy will be considerably higher.

6. It has incidentally been observed that the mixture has a fair degree of efficacy against *Haemonchus contortus* and *Moniezia expansa*.

7. Measures for treatment and prevention of oesophagostomiasis by means of a suitable remedy under South African conditions are outlined.

 ADDENDUM.

The following information is available with regard to the test referred to above:—

Reports have been received on 94,263 sheep treated by 103 farmers. The total losses registered after treatment are 231 or 0.245 per cent. Most of the sheep were in poor condition and were grazing on dry pasture. In some cases the deaths were definitely due to the treatment; one flock which was affected had been starved; in a few cases the powder was inhaled and in some other cases the deaths were due to weakness and would probably have occurred in any case. In two cases, accounting for losses of 30 and 28 sheep respectively, the sheep were receiving fairly large rations of crushed maize. From

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observations made it appears that crushed maize or other grain frequently reaches the abomasum from the fore-stomachs and there is then a tendency towards retention of the abomasal contents until the grain has been softened and broken up. This dietetic factor seems to be of considerable importance in causing stagnation in the abomasum, which is dangerous in connection with this treatment.

A few farmers were not satisfied with the efficacy of the remedy since only a small number of worms were observed to be passed or some sheep killed after treatment still had a residual infection. The large majority, however, reported that large numbers of worms had been passed and they were well pleased with the result. In several cases it was reported that deaths due to oesophagostomiasis stopped immediately and with few exceptions the reports contain the statement that the sheep improved markedly after treatment. It was also noted in several cases that the animals started to feed much better than before from the day after the administration of the second dose.

From the experience gained the following appear to be important points to observe and these are being incorporated in the directions for use of further issues of the remedy.

1. The sheep should get no grain ration or salt lick from two days before until a day after treatment.
2. The sheep should have access to water immediately before being dosed.
3. If the pasture is dry it is very desirable to allow green food for a few hours before each dosing.
4. In order to prevent inhalation the powder should be administered immediately after the bluestone solution, so that it reaches the pharynx when the sheep has closed the glottis in preparation for swallowing the bluestone solution.

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APPENDIX.

NODULAR WORM REMEDY: DIRECTIONS FOR USE.

The remedy is issued in tins which contain a sufficient quantity for single dosing of 100 sheep over 18 months, or 140 sheep between 6 and 18 months, or 250 lambs of 3 to 6 months.

METHOD OF TREATMENT.

The sheep must not be kept from food or water before treatment and may graze again immediately after treatment but should have no water for 1-2 hours. They may also be treated towards evening and then remain in the kraal overnight.

Suckling lambs must not have a drink four hours before and four hours after treatment.

The efficacy of the remedy depends on its being swallowed directly into the fourth stomach, and this is brought about by administering a small quantity of bluestone solution immediately before the remedy. The moment the bluestone reaches the throat of the sheep the large stomach closes and remains closed for 15 seconds.

The treatment therefore consists of two parts as follows:—

1. The spoon marked X is filled with a 10 per cent. bluestone solution—to prepare this dissolve 1 pound bluestone in 1 gallon of water, or 2 ounces to 1 pint water, or 2½ ounces to 1 bottle water—open the sheep's mouth well and pour the bluestone alongside of the tongue so that it runs down into the throat. For small lambs a ¾ spoonful is sufficient.
2. The correct measuring spoon is filled with the remedy beforehand and is kept ready to be emptied on to the back of the tongue immediately after giving the bluestone, without having closed the sheep's mouth. Directly after the administration of the powder the mouth is closed and the sheep released.

The sheep have to get a *double dose* of the remedy and are therefore treated in the same way on two successive days.

Before using the remedy it is desirable to empty the contents of each tin into a suitable receptacle and to stir it well.

A quantity of the powder is then placed into the tin with the crossbeam (as used with Government Wireworm Remedy and obtainable from the Laboratory). The correct measuring spoon is filled lightly, without pressing against the side of the tin, and scraped off level against the underside of the crossbeam.

The remedy is administered by means of the measuring spoon. Should the spoon become wet and the powder cling to it, it must be wiped. The following are the correct doses:—

For lambs 3 to 6 months, spoon No. 1.

For lambs over 6 to 18 months, spoon No. 2.

For sheep over 18 months, spoon No. 3.

Sheep which are very weak or small for their age should get a smaller dose on the second day, e.g. a 2-tooth sheep first day spoon No. 3 and second day spoon No. 2. Weak lambs are dosed once only. Lambs under 4 months should not be dosed unless it is really necessary.

RESULTS OF TREATMENT.

The remedy causes much slime to be formed in the large intestine and the droppings therefore often contain much slime after treatment and have a blue-green colour due to the remedy. It is expected that one treatment (double dosing) will remove at least three-fourths of the worms and many sheep will be clean. It has also been noticed that the remedy is effective against wireworms and tapeworms in sheep.

WHEN AND HOW OFTEN TO TREAT.

In order to understand this properly, the life-cycle of the nodular worm must be explained. The worm eggs are passed in the droppings of the sheep and hatch in the pasture if it is moist and warm. The small worms are ready to infect sheep after a week and can live in the pasture for several months. In winter when it is dry the pasture becomes clean, because the eggs and young worms are killed by the prolonged drought. Infested sheep are the source of infection for the next summer. (They should therefore be cleaned during the winter.)

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When the young worms are swallowed by a sheep they bore into the wall of the intestine, causing the well-known nodules to develop. Here they may stay for 5 days to 3 months, then they return to the inside of the gut, pass to the large bowel where they grow adult and begin to lay eggs after about 5 weeks. (One treatment therefore does not clean sheep, since the young worms in the nodules are not killed.)

The sheep therefore infect the pasture when the rainy season starts and they themselves become further infected during the summer. When the pasture becomes dry and feeding poor about April, the sheep begin to die of the worms which they harbour. (They should therefore be treated before this time arrives.)

The following is therefore recommended:—

1. Badly infested sheep may be treated at any time of the year to cure them and three treatments with intervals of 4-6 weeks are necessary. (It will, however, do no harm if the sheep are treated a few times at intervals of 2 weeks.)
2. Under ordinary circumstances it will suffice to treat every six weeks from January or February until July. The first few treatments are to cure the sheep before they should die in April-May. The further treatments are to clean them thoroughly during the winter, so as to remove the source of infection for the following year.
3. It is expected that after a few years of treatment as indicated under 2, the nodular worm as a pest will disappear. In order to prevent further trouble it is strongly recommended that sheep should then still be dosed annually three times with intervals of six weeks between the months of April and July.

Wireworms.—Sheep may be dosed for wireworms a fortnight before or after dosing for nodular worm. Since the nodular worm remedy kills wireworms fairly effectively, the usual treatment for wireworms can be carried out under scheme 2 outlined above each third week after a treatment for nodular worm. At other times sheep should be dosed for wireworms throughout the year at intervals of 3 weeks, or in very wet summer months every 2 weeks, using the ordinary wireworm remedies.

SPECIAL MEASURES.

Ewes.—It is not advisable to treat ewes from about a month before lambing up to about a fortnight thereafter. Pregnant ewes must be handled carefully.

The sheep should not be chased about unduly during or after dosing and it is desirable to use a suitable crush for dosing.

As the remedy is poisonous it should be handled carefully and sheep should not get more than the doses recommended.

LASTING PROPERTIES OF THE POWDER.

The powder does not decompose or lose strength, but it is advisable to keep the tins well closed and to store them in a dry place.

PRICE-LIST.

Tins of powder containing 100 single doses for sheep over 18 months, each 1s. 3d. (for one treatment of 100 adult sheep two such tins are required).

Measuring spoons—set of 4 (X, 1, 2 and 3), 5s.; single spoons, 1s. 3d. Measuring bowl with crossbeam, 6d. each. Post or rail-free to any post of the Union.

These articles are delivered only for cash or c.o.d. by rail or post. Orders to be addressed to the Director of Veterinary Services, P.O. Onderstepoort, Pretoria.

All Laboratory products are carefully tested before issue but are issued solely at buyer's risk. The Government cannot be held responsible for any losses or accidents which might possibly occur after their use.

N.B.—Empty tins should not be returned.