# THE PATHOLOGIGAL PHYSIOLOGY OF HELMINTH INFESTATIONS. II. OESOPHAGOSTOMUM COLUMBIANUM

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# INTRODUCTION

Recent surveys have shown *Oesophagostomum columbianun* to be the most widespread nematode parasite of sheep in South Africa (Muller, 1962, 1964; Barrow, 1964; Rossiter, 1964; Viljoen, 1964). These workers and Reinecke (1964) suggested methods of control for this parasite based on its seasonal incidence. Veglia (1923, 1928) gave a detailed description of the life cycle and the disease caused in sheep and Fourie (1936) studied the pathological anatomy of oesophagostomiasis extensively.

The present experiments were undertaken in order to determine the effects of the parasite on the physiological processes of sheep.

#### Methods

Merino and Dorper (Dorset Horn  $\times$  Black Head Persian) sheep, bred and reared worm-free were used in these experiments.

Blood analyses and faecal egg counts were done as described by Horak & Clark (1964). Infestation, recovery and counting of worms were carried out as reported by Reinecke, Horak & Snijders (1963).

The sheep were housed separately and lucerne hay and water were offered *ad lib*. to all infested sheep. Daily feed and water consumption and rectal temperature and weekly body weight were recorded.

Six sheep were divided into pairs. One member of each pair was infested while the other member was kept as a worm-free control which received an amount of feed equal to that eaten by its infested partner the previous day (Bremner, 1961).

# EXPERIMENTAL ANIMALS

Oesophagostomiasis can be divided into acute and chronic phases. The acute disease is caused by migrating larval stages before reaching patency, whereas the chronic disease is caused by larvae and adult worms. For clarity the sheep are therefore grouped according to symptoms.

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Acute disease..... Single massive infestation. Three sheep.

- Sheep 1: Uninfested control, feed controlled by the previous day's intake of sheep 2.
- Sheep 2: Infested with 8,000 larvae. Died 23 days later.

Sheep 3: Infested with 12,000 larvae. Died 18 days later.

*Chronic disease*..... Single massive infestation followed by an anthelmintic. Four sheep.

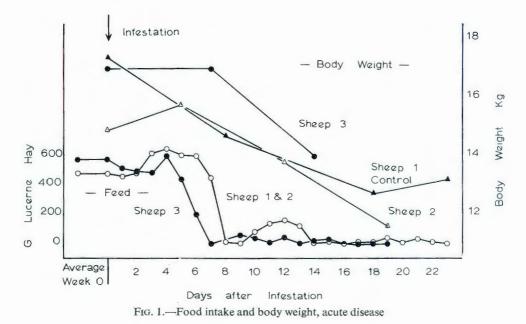
- Sheep 4 and 5: Infested with 8,000 larvae each, followed two days later by thiabendazole at 100 mg/Kg live weight. Sheep 4 killed 107, and sheep 5 killed 49 days after infestation respectively.
- Sheep 6 and 7: Infested with 12,000 larvae each, followed two days later by thiabendazole at 100 mg/Kg live weight. Both killed 84 days after infestation.

*Chronic disease with controls*..... Single massive infestation followed by an anthelmintic.

Sheep 8 and 10: Uninfested controls, feed controlled by previous day's intake of sheep 9 and 11 respectively.

Sheep 9 and 11: Infested with 8,000 larvae each, followed two days later by thiabendazole at 100 mg/Kg live weight. Sheep 11 killed 63 days after infestation.

Reinfestation..... Single massive infestation followed by challenge. One sheep. Sheep 12: Infested with 8,000 larvae followed 54 days later by 8,000 larvae. Killed 84 days later.



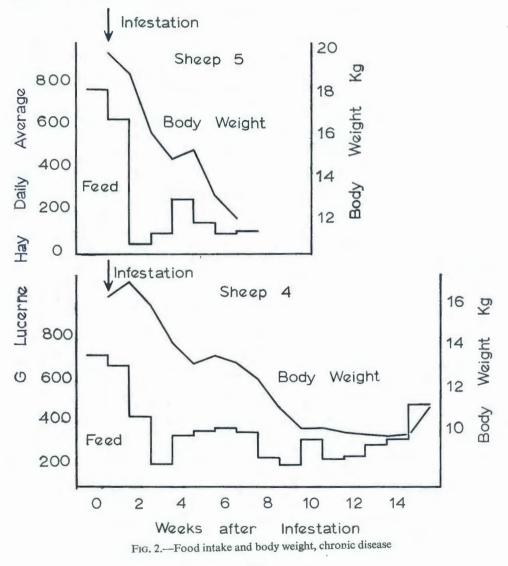
# RESULTS

These will be discussed under the following headings:----

## Clinical Observations

# (1) Food and water intake and body weight

Acute disease: A marked decrease in food intake occurred seven days after infestation in sheep 2 and at five days in sheep 3; both then stopped eating for one or two days followed by a slight increase in appetite to fall again to a period of no or very little intake (Fig. 1). The intake of sheep 1 was controlled by that of sheep 2 and is shown with that of sheep 2 in Fig. 1.

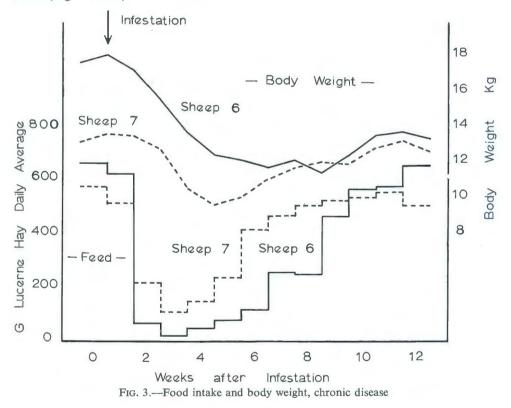


141

The body weights of all three sheep declined during the second week of infestation The total loss in body weight of sheep 1 was slightly greater than that of sheep 2, its infested partner (Fig. 1).

*Chronic disease:* The feed intake and body weights of sheep 4 and 5 are shown in Fig. 2 and those of sheep 6 and 7 in Fig. 3. All the animals showed a marked decrease in food intake during the second and third week of infestation followed by a slight increase and then further decrease in sheep 4 and 5 with a terminal increase in sheep 4. A gradual improvement in intake was noted in sheep 6 and 7 starting during the fourth week of infestation.

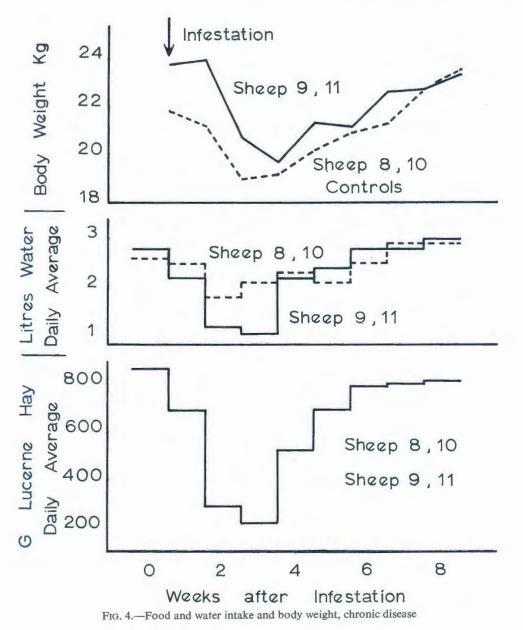
The body weights of the various sheep closely paralleled their food intake but the improvement in body weight of sheep 4, 6 and 7 was more gradual than that in food ntake (Fig. 2 and 3).



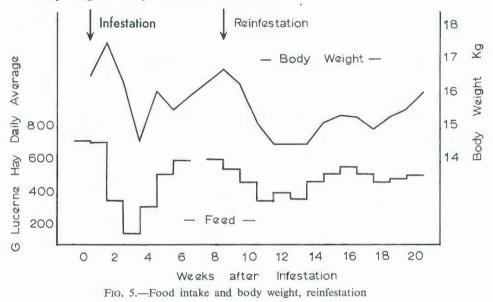
*Chronic disease with controls:* Feed and water intake and body weight of all four sheep were markedly reduced during the second and third week of infestation, followed by a gradual increase. During the period of minimum food intake the uninfested sheep (sheep 8 and 10) drank considerably more water than the infested sheep (Fig. 4)

At the termination of this experiment the controls had gained weight whereas the infested sheep were still below their initial body weights (Fig. 4).

I. G. HORAK & R. CLARK



*Reinfestation:* The food intake and body weight of sheep 12 are shown in Fig. 5. After initial infestation feed consumption decreased during the second and third week followed by a steady increase. A decrease in food intake was again noted during the second and third week after reinfestation followed by a more gradual increase than previously.



Body weight closely followed food intake.

#### (2) Rectal temperature

The rectal temperatures of some of the sheep were taken daily and the weekly averages for these sheep are shown in Fig. 6. An increase in temperature occurred during the second or third week of infestation and reached a peak during the third or fifth week followed by a sharp decline. The uninfested controls (sheep 1, 8 and 10) showed no temperature rise.

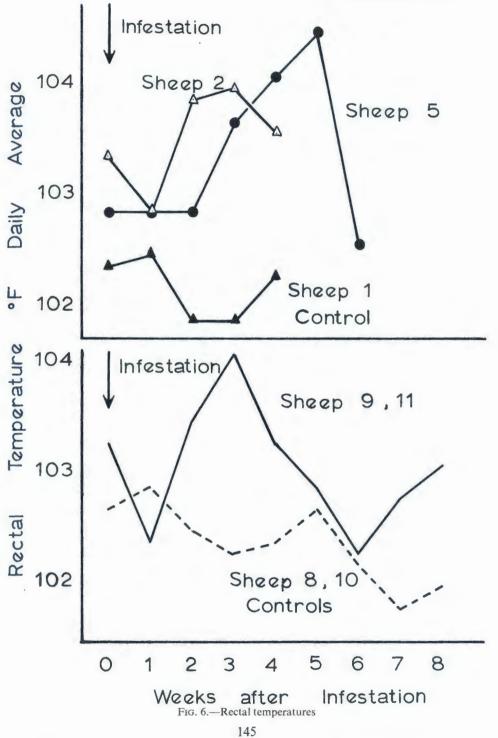
# (3) Diarrhoea

Diarrhoea occurred in all the infested sheep. In most cases this diarrhoea was preceded by a period lasting a few days during which the faeces were extremely dry and lacked the normal shiny mucous coating.

Diarrhoea was noted as early as the 10th or as late as the 20th day of infestation (sheep 11 and 4 respectively). In the other sheep it commenced between the 12th and 16th day. The nature of the excreta varied considerably being either putty-like, fluid, fluid-mucoid, mucopurulent or green and putrid-smelling. This diarrhoea persisted intermittently until death or slaughter.

#### (4) Colic

Sheep 3 and 5 first showed symptoms of abdominal colic on the seventh and 16th days of infestation respectively. The fore and hind limbs were placed far apart and the sheep appeared to be stretching; these symptons lasted for several days, and were similar to those associated with intussusception in sheep.



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# Clinical chemistry

(1) Acute disease

The clinical chemistry findings for each of the three sheep are presented in Table 1 and Fig. 7.

TABLE 1.	-Clinical	chemistry	findings	of	sheep	with	acute	oesophagostomiasis
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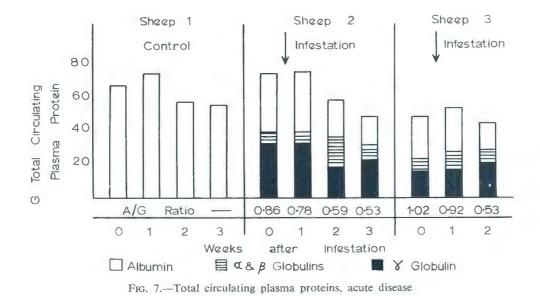
Weeks after infestation	0	1	2	3
Packed Cell Volume %	31 28 33	30 27 33	33 27 39	37 25
Haemoglobin gm %	11·1 9·8 12·4	12·2 9·5 12·7	$ \begin{array}{c} 12 \cdot 1 \\ 8 \cdot 6 \\ 13 \cdot 8 \end{array} $	13·5 9·1
Plasma Volume (Litre)	1.00 0.97 0.88	$1 \cdot 02 \\ 1 \cdot 17 \\ 0 \cdot 96$	0.89 1.02 0.77	0.82 0.90
Circulating Red Blood Cell Volume (Litre)	$0.45 \\ 0.38 \\ 0.43$	$0.43 \\ 0.43 \\ 0.47$	0·44 0·38 0·49	0·48 0·29
Total Plasma Protein gm %	$6 \cdot 82 \\ 6 \cdot 32 \\ 5 \cdot 64$	$7 \cdot 38 \\ 6 \cdot 50 \\ 5 \cdot 64$	$6 \cdot 50 \\ 5 \cdot 80 \\ 5 \cdot 80$	6·82 5·48
Blood Sugar mg %	75 51 64	56 44 30	51 32 22	49 27 —
Plasma Ca mg %	12·9 11·5 10·9	10·6 11·8	9·1 9·3 9·4	9·4 8·3
Plasma Inorganic PO <sub>4</sub> mg %	4·2 5·1 5·5	$7 \cdot 4$ $5 \cdot 6$ $4 \cdot 4$	$ \begin{array}{r} 12 \cdot 8 \\ 6 \cdot 9 \\ 4 \cdot 3 \end{array} $	9·3 5·1

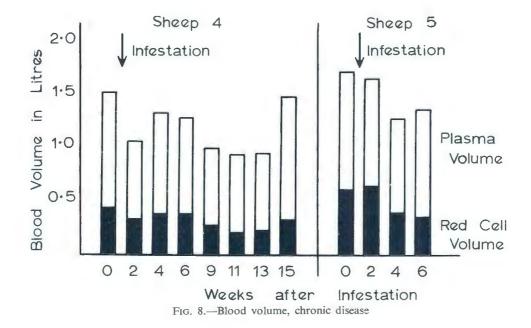
The figures for sheep 1, 2 and 3 are given consecutively under each heading.

Sheep 1 and 3 showed a rise in packed cell volume and haemoglobin concentration. This was due to plasma volume shrinkage and an increase in the absolute volume of circulating red blood cells during the same period. Sheep 2 showed a decline in all the above mentioned factors (Table 1).

Although the total plasma protein concentration of sheep 1 and 3 remained fairly constant and that of sheep 2 decreased slightly (Table 1), the total circulating plasma proteins of all three animals showed a noticeable drop (Fig. 7). In those sheep (2 and 3), in which the proteins were fractionated, this decrease was due mainly to a drop in plasma albumin. Plasma gamma globulin fell in sheep 2 while it rose in sheep 3. This drop in plasma albumin, plus the rise in plasma gamma globulin in sheep 3, resulted in the albumin/globulin ratio in these sheep dropping from 0.86 and 1.02 respectively to 0.53.

Blood sugar and plasma calcium concentration decreased in all three sheep. These changes were associated with reduced food intake and the drop in plasma albumin respectively.





## (2) Chronic disease

The clinical chemistry findings in the four sheep are presented in Table 2 and Fig. 8, 9 and 10.

Weeks After Infesta- tion	0	2	4	6	9	11	13	15
Packed Cell Vol- ume %	30 37 34 30	31 39 39 28	29 30 30 26	30 26 32 27	28 27 32	22 30 32	24	22
Haemoglobin gm %	$   \begin{array}{r}     11 \cdot 1 \\     13 \cdot 5 \\     11 \cdot 7 \\     10 \cdot 3   \end{array} $	$   \begin{array}{r}     11 \cdot 4 \\     14 \cdot 9 \\     14 \cdot 9 \\     10 \cdot 3   \end{array} $	$ \begin{array}{c} 11 \cdot 1 \\ 12 \cdot 4 \\ 11 \cdot 3 \\ 10 \cdot 3 \end{array} $	$ \begin{array}{r}     \hline     10\cdot 3 \\     11\cdot 3 \\     9\cdot 3 \end{array} $	$ \begin{array}{r} 10\cdot 8 \\ \hline 10\cdot 3 \\ 11\cdot 6 \end{array} $	8.8 11.6 11.0	9·2	8.9
Total Plasma Protein gm %	5.64 5.64 5.80 6.32	$5 \cdot 80$ $5 \cdot 64$ $6 \cdot 16$ $6 \cdot 32$	$6 \cdot 00$ $5 \cdot 64$ $5 \cdot 80$ $6 \cdot 00$	$5 \cdot 53$ $5 \cdot 46$ $5 \cdot 10$ $5 \cdot 80$	$ \begin{array}{r} 4\cdot 40 \\ - \\ 6\cdot 16 \\ 5\cdot 80 \end{array} $	4·56 	4·40 	4.56
Blood Sugar mg %	59 86 68 58	36 51 29 40	$\begin{array}{c c} 41 \\ \hline 47 \\ 34 \end{array}$	33 47 48 33	53 47 41	45 49 47	71	59
Plasma Ca mg %	9.7 12.3 11.5 12.1	$   \begin{array}{r}     11 \cdot 1 \\     10 \cdot 6 \\     10 \cdot 0 \\     10 \cdot 0   \end{array} $	11·9 11·2	$   \begin{array}{r}     11 \cdot 6 \\     9 \cdot 4 \\     10 \cdot 0 \\     11 \cdot 3   \end{array} $	$ \begin{array}{r} 10 \cdot 6 \\ 11 \cdot 2 \\ 10 \cdot 6 \end{array} $	$9 \cdot 7$ $11 \cdot 5$ $12 \cdot 6$		11.2
Plasma Inorganic PO <sub>4</sub> mg %	$5 \cdot 3$ $3 \cdot 2$ $4 \cdot 6$ $6 \cdot 4$	$4 \cdot 0$ $6 \cdot 2$ $4 \cdot 8$ $7 \cdot 3$	$ \begin{array}{r} 4\cdot 4 \\ \overline{} \\ 5\cdot 9 \\ 7\cdot 2 \end{array} $	$   \begin{array}{r}     4 \cdot 0 \\     6 \cdot 8 \\     4 \cdot 7 \\     4 \cdot 6   \end{array} $	$     3 \cdot 5     \overline{3 \cdot 3}     5 \cdot 4 $	$ \begin{array}{r} 4 \cdot 7 \\ \overline{5 \cdot 4} \\ 6 \cdot 0 \end{array} $	5·4 	2·3
Eosinophile Counts/ cub mm		25 0 0	150 1,200 0 150	475 500 375 625	275 225 925	50 150 800	50	250
Average Weekly E.P.G. Counts	0 0 0 0	0 0 0 0	0 0 0 0	20 (41) 100 (45) 0 40 (38)	4,625 250 (56) 775	6,300 580 1,160	9,650	4,267
Sheep 12	0	0	0	167 (37)	1,200	1,000	2,700	1,775

TABLE 2.—Clinical chemistry findings of sheep with chronic oesophagostomiasis

The figures for sheep 4, 5, 6 and 7 are given consecutively under each heading. Figures in brackets indicate the day on which the first egg was seen in the faeces

Sheep 4 and 5 showed a decrease in packed cell volume, haemoglobin concentration, absolute volume of circulating erythrocytes and plasma volume during the ninth and fourth week of infestation respectively (Table 2 and Fig. 8). There was no recovery in any of these factors in either of the sheep, with the exception of a terminal rise in the volume of circulating erythrocytes and plasma volume in sheep 4.

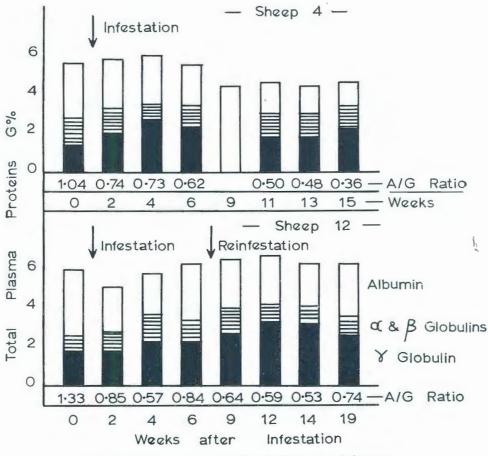
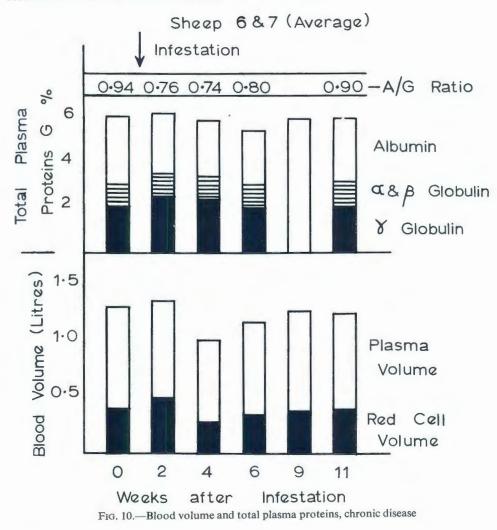


FIG. 9.-Total plasma proteins, chronic disease and reinfestation

From the ninth week of infestation sheep 4 exhibited a drop in total plasma protein concentration. This decrease was entirely due to a drop in plasma albumin concentration, from 2.8 gm per cent initially to 1.2 gm per cent finally. An increase in plasma gamma globulin concentration took place at four weeks but decreased thereafter to rise again at the 15th week of infestation. While the albumin/globulin ratio dropped from an initial value of 1.04 to a final value of 0.36 (Fig. 9).

Sheep 6 and 7 showed a decrease in packed cell volume, absolute volume of circulating erythrocytes and plasma volume during the fourth week and a slight drop in total plasma protein concentration during the sixth week of infestation. All these factors, however, soon returned to normal (Table 2 and Fig. 10). No marked changes were noted in the individual plasma protein fractions.

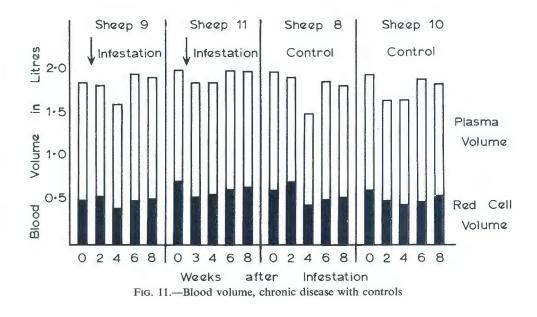
High eosinophile counts were noted in all four sheep from the fourth to the sixth week of infestation onwards.

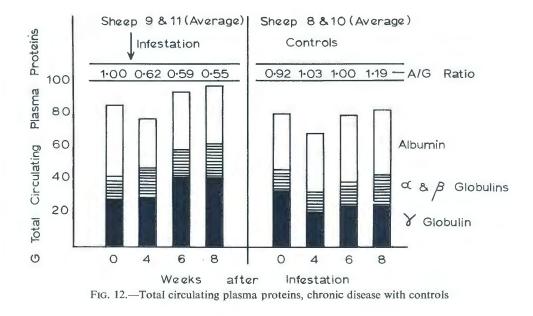


# (3) Chronic disease with controls

The clinical chemistry findings for each of the four sheep are presented in Table 3 and Fig. 11 and 12.

The drop in packed cell volume, haemoglobin concentration, absolute volume of circulating erythrocytes, plasma volume and total circulating plasma proteins that occurred in the infested sheep (sheep 9 and 11) was paralleled by similar changes in the controls (sheep 8 and 10) (Fig. 11). Marked differences, however, did occur in the total circulating plasma protein fractions. Whereas a decrease in total circulating gamma globulin took place in the infested sheep no such changes occurred in the controls (Fig. 12). There were also marked differences between the eosinophile counts of the infested and control sheep (Table 3).





Weeks After Infestation	0	2	3	4	6	8
Packed Cell Volume %	31 28 33 31	38 30 35 36	35 26 30 30	30 26 28 31	27 26 26 31	30 27 31 32
Haemoglobin gm %	$   \begin{array}{r}     11 \cdot 9 \\     10 \cdot 5 \\     12 \cdot 7 \\     11 \cdot 5   \end{array} $	$   \begin{array}{r}     13 \cdot 8 \\     11 \cdot 1 \\     13 \cdot 5 \\     14 \cdot 0   \end{array} $	$     \begin{array}{r}       12 \cdot 7 \\       10 \cdot 3 \\       11 \cdot 9 \\       11 \cdot 9     \end{array}   $	$   \begin{array}{r}     11 \cdot 9 \\     10 \cdot 5 \\     11 \cdot 1 \\     12 \cdot 7   \end{array} $	$   \begin{array}{r}     10 \cdot 0 \\     10 \cdot 3 \\     9 \cdot 5 \\     11 \cdot 3   \end{array} $	$   \begin{array}{r}     12 \cdot 7 \\     11 \cdot 4 \\     12 \cdot 4 \\     13 \cdot 0   \end{array} $
Total Plasma Protein gm %	$6 \cdot 50 \\ 6 \cdot 00 \\ 4 \cdot 92 \\ 6 \cdot 00$	$     \begin{array}{r}       6 \cdot 32 \\       5 \cdot 80 \\       5 \cdot 46 \\       6 \cdot 00     \end{array} $	$6 \cdot 00 \\ 5 \cdot 80 \\ 5 \cdot 46 \\ 5 \cdot 64$	$6 \cdot 50 \\ 6 \cdot 16 \\ 5 \cdot 64 \\ 6 \cdot 16$	$     \begin{array}{r}       6 \cdot 00 \\       6 \cdot 82 \\       5 \cdot 46 \\       6 \cdot 00     \end{array} $	6.68 7.38 6.16 6.68
Blood Sugar mg %	59 77 72 63	41 59 49 48	37 40 60 38		47 50 58 47	45 48 52 48
Plasma Ca mg %	$     \begin{array}{r}       11 \cdot 9 \\       11 \cdot 6 \\       11 \cdot 1 \\       11 \cdot 6     \end{array} $	$     \begin{array}{r}       11 \cdot 8 \\       10 \cdot 6 \\       10 \cdot 6 \\       11 \cdot 2     \end{array} $	7.8 9.7 10.6 10.3		$   \begin{array}{r}     10 \cdot 6 \\     11 \cdot 8 \\     10 \cdot 9 \\     11 \cdot 2   \end{array} $	$     \begin{array}{r}             11 \cdot 8 \\             10 \cdot 6 \\             11 \cdot 2 \\             10 \cdot 6         \end{array}     $
Plasma Inorganic PO <sub>4</sub> mg %	$5 \cdot 1$ $4 \cdot 0$ $3 \cdot 9$ $4 \cdot 7$	7.8 3.7 7.7 5.1	$   \begin{array}{r}     11 \cdot 1 \\     7 \cdot 0 \\     4 \cdot 3 \\     4 \cdot 8   \end{array} $		$5 \cdot 7$ $5 \cdot 0$ $5 \cdot 4$ $5 \cdot 1$	$7 \cdot 2 \\ 6 \cdot 2 \\ 6 \cdot 8 \\ 7 \cdot 0$
Eosinophile Counts/cub mm			50 225 25 2,000	50 700 25 425	50 2,700 25 650	50 2,500 50 1,550
Average Weekly E.P.G. Counts	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 50 (41)	0 850 (45) 0 500

 TABLE 3.—Clinical chemistry findings of sheep with chronic oesophagostomiasis and their uninfested controls

The figures for sheep 8 and 9 and sheep 10 and 11 are given consecutively under each heading. (Sheep 8 and 10 = Uninfested controls). Figures in brackets indicate the day on which the first egg was seen in the facees.

The average initial and lowest figures for total blood volume, plasma volume and circulating erythrocyte volume for the six chronically infested sheep (sheep 4 to 7, 9 and 11) as well as those for the two control sheep (sheep 8 and 10) are given in Table 4. Marked actual and percentage decreases in all three factors occurred in both the infested and control sheep.

#### I. G. HORAK & R. CLARK

Chronically Infested Sheep									
Blood Volume	Inital B1 vol (Litre)	At lowest B1 vol (Litre)	%Change						
Plasma Volume	1.12	0.94	-16.1						
Circulating Red Blood Cell Volume	0.51	0.37	-27.5						
Total Blood Volume	1.63	1.31	-19.6						

 TABLE 4.—Blood constituents of chronicially infested sheep and uninfested control sheep

#### Uninfested Control Sheep

Plasma Volume	1.43	1.08	-24.5
Circulating Red Blood Cell Volume	0.66	0.46	-30.3
Total Blood Volume	2.03	1.54	-26.3

B1 vol = Blood volume.

Similarly the average figures for the various plasma protein fractions and albumin/globulin ratio for the same sheep are given in Table 5. Whereas a marked decrease in plasma albumin concentration and albumin/globulin ratio and increase in alpha, beta and gamma globulin concentration characterizes the changes in the infested sheep, a constant albumin/globulin ratio was maintained in the controls despite variations in the plasma protein fractions.

#### (4) Reinfestation

After initial infestation sheep 12 exhibited a drop in packed cell volume, haemoglobin concentration, absolute volume of circulating erythrocytes and plasma volume. At the time of reinfestation these factors had not quite recovered and decreased again as a result of the larval challenge to increase slightly at the termination of the experment.

The fluctuations in the plasma protein fractions of this sheep are shown in Fig 9. Plasma albumin decreased during the second week after initial infestation and remained low until the sixth week, when a slight improvement was noted. After reinfestation plasma albumin again decreased to recover only at the termination of the experiment.

Four weeks after infestation there was a slight increase in plasma gamma globulin which was repeated to a larger extent after challenge. This taken in conjunction with the decrease in plasma albumin resulted in an albumin/globulin ratio of 0.53(Fig. 9).

	Chronically Infested Sheep							
Protein Fractions	Initial gm %	At Lowest T.P.P. gm	% Change	Final T.P.P. gm	% Change			
Albumin	2.90	2.21	-23.8	2.21	-23.8			
$\alpha \& \beta$ Globulin	1.04	1.21	+16.3	1 · 27	+22.1			
γ Globulin	1.96	2.00	+ 2.0	2.53	+29.1			
Т.Р.Р	5.90	5.42	- 8.1	6.01	+ 1.9			
A/G Ratio	0.97	0.69		0.58				
		Uninfes	ted Contro	l Sheep				
Albumin	2.80	2.92	+ 4.3	3.10	+10.7			
$\alpha \& \beta$ Globulin	0.91	1.07	+17.6	1.44	+58.2			
y Globulin	2.00	1.74	-13.0	1.88	- 6.0			
Т.Р.Р.	5.71	5.73	+ 0.4	6.42	+12.4			
A/G Ratio	0.96	1.04		0.93				

# TABLE 5.—Protein fractions of chronically infested sheep and uninfested control sheep

T.P.P. = Total plasma proteins

A/G Ratio = Albumin/Globulin Ratio

# Faecal examinations

These are summarized at the foot of Tables 2 and 3. The first eggs were seen in the faeces 37 to 56 days after infestation. In sheep 4, 5, 6 and 12 the number of eggs per gram of faeces was still rising by the 12th to 13th week of infestation although only in sheep 4 and 12 did these counts exceed 2,000 eggs per gram.

# Pathological anatomy

The following macroscopic changes were noted: Emaciation, peritonitis, perforation of the intestine with extensive adhesions between the coils of the colon, between the small intestine and the caecum; serous atrophy of the mesenteric fat, oedema of the mesenteric lymph nodes; thickened intestinal wall and numerous caseous and calcified nodules in the wall of the large and small intestine, purulent nodules in the intestinal lymph nodes, kidneys, liver and lungs.

#### Worms recovered post mortem

Results are summarized in Table 6.

Sheep No.			O. colui	mbianum		
	Age of Infestation in days					
		4th (Intestinal Wall)	4th (Intestinal Lumen)	5th (Lumen)	Adult (Lumen)	Total
2* 3* 4 5 6 7 11 12	23 18 107 49 84 84 63 84–138	195 5 0 100 57 103 675 50	897 589 0 341 0 0 40 10	0 0 21 220 80 23 30 76	0 0 319 20 340 278 278 274 785	1,092 594 340 681 477 404 1,019 921

# TABLE 6.—Worms recovered at autopsy

\*Died, acute oesophagostomiasis

5th = 4th Moult and 5th stage worms before copulation

No cross-infestation with other helminths was present. Fourth stage worms predominated in sheep 2 and 3 which died 23 and 18 days after infestation respectively, whereas, with the exception of sheep 11, fifth stage and adult worms were the most numerous in the other sheep.

#### DISCUSSION

# Clinical observations

Anorexia was found to be the most important symptom in sheep with oesophagostomiasis and confirms Bremner's (1961) observations in cattle. It has been reported in fascioliasis (Sinclair, 1962), paramphistomiasis (Horak & Clark, 1963), ostertagiasis (Horak & Clark, 1964; Horak, Clark & Botha, 1965), and trichostrongylosis (Gordon, 1950). But while in the latter infestations it plays a secondary role, in oesophagostomiasis it is of primary importance, many other changes being due to this anorexia.

About six to eight days after single infestations massive migration of fourth stage larvae from the intestinal wall to the intestinal lumen occurs (Veglia, 1923; Fourie, 1936; Reinecke, *et al.* 1963); at the same time anorexia commences (Fig. 1 to 5) The cause of this anorexia may, therefore, be due to the intestinal discomfort and damage caused by the migrating larvae.

Clark & Quin (1949) found that sheep on a low food intake usually consume correspondingly less water; this observation was confirmed as the water intake of the infested sheep decreased with the decreased food intake. The water intake of the uninfested control sheep (sheep 8 and 10), which were fed the same amount of lucerne hay as their infested partners (sheep 9 and 11) respectively, did not decrease to the same extent as did that of the infested sheep (Fig. 4). This was probably due

to the fact that the anorexia in the infested sheep was due to loss of appetite, whereas that in the controls was artificially enforced and hence these sheep drank more water to supplement the volume of their total intake.

In all the sheep decrease in body weight closely followed decreased food and water consumption. In this respect decreased intestinal contents due to the decreased intake played a major role in body weight decrease. By the end of the third week of infestation the average body weight of sheep 9 and 11 had decreased by  $4 \cdot 2$  Kg, whereas their average total daily intake of food and water had increased by  $2 \cdot 4$  Kg, thus accounting for 57 per cent of the loss in body weight (Fig. 4).

This dependence of body weight on intestinal contents has an important bearing on the method by which plasma volume is expressed. The absolute plasma volume is not affected, but if plasma volume is expressed as ml per Kg of body weight, the drop in total body weight due to the decreased intestinal contents can give an artificially high value.

In no case did any of the infested sheep regain their original body weight during the course of a particular experiment. The uninfested controls, sheep 8 and 10 however, kept on the same hay intake as sheep 9 and 11 respectively, gained an average of  $2 \cdot 2$  Kg by the end of that experiment, while sheep 9 and 11 were an average of  $0 \cdot 4$  Kg lighter than their original weight. It would, therefore, appear that infestation in some way interferes with the absorption of food or its metabolism resulting in a decreased conversion rate. The prolonged effect of this infestation on body weight is of great economic importance in fat-lamb production, where these animals have to reach a marketable weight at an early age.

The increase in rectal temperature during oesophagostomiasis has been recorded by Veglia (1928) and it would appear to be due to the damage caused by the migrating fourth stage larvae and to bacterial invasion of these lesions.

The severity of the diarrhoea, which occurred in all the infested sheep, determined the severity of the ensuing chronic disease. In sheep 4 where diarrhoea was delayed until the 20th day and was never marked, a severe chronic disease developed, which only appeared to recover by the 15th week of infestation. In the other sheep with chronic infestations the diarrhoea commenced earlier and was more severe and recovery took place sooner. This was probably due to the fact that during the course of the disease many of the worms are excreted by the diarrhoea (Reinecke *et al.*, 1963; Bremner, 1961; Veglia, 1923). This agrees with the observations of Sarles (1944) who recorded an inverse ratio between the number of infective larvae dosed and the number of adults recovered.

Veglia (1928) and Fourie (1936) suggested that *O. columbianum* infestation in sheep could result in intussusception or "reksiekte". The symptoms of stretching and colic seen in the present experiments would suggest that intussusception of a temporary nature had been produced or that the intestinal adhesions had produced similar symptons. The rectal prolapse seen in sheep 5 seemed to be a direct result of the diarrhoea and intestinal damage caused by the larvae.

#### Clinical Chemistry

The changes in packed cell volume, haemoglobin concentration, absolute volume of circulating erythrocytes, plasma volume, total plasma protein concentration and total circulating plasma proteins that occurred in the chronic disease, appeared to be dietetic in origin as similar changes occurred in the uninfested controls (sheep 8 and 10). Sheep 4, however, after a slight decrease in the above factors coinciding with the initial anorexia, developed a fairly severe anaemia and hypoproteinaemia during the latter stages of the disease which could have been parasitic in origin. Marked differences, however, between the infested and control sheep were apparent in plasma albumin concentration, plasma gamma globulin concentration and albumin/globulin ratio. The increase in plasma gamma globulin was particularly noticeable after reinfestation.

As in ostertagiasis (Mulligan, Dalton & Anderson, 1963) the plasma albumins could be lost by seepage into the lumen of the intestine via the lesions caused by the migrating fourth stage larvae. The reason for the increase in plasma gamma globulin is unknown, but may be related to antibody production.

Bremner (1961) studied the effects of *O. radiatum* in cattle using three pairs of calves. One member of each pair was infested while the other member received the same amount of feed as its infested partner had eaten the previous day. He recorded a decrease in packed cell volume in both the infested and control calves, although that in the former was far more severe. The infested calves also showed a drop in red cell counts, haemoglobin and total plasma protein concentrations as well as a decrease in total circulating plasma proteins which the controls did not. The decrease in total plasma protein concentration increased again it was mainly due to the globulin fractions. When this concentration increased again it was mainly due to the globulin fraction resulting in an albumin/globulin ratio in the infested calves much lower than that in the controls. The chief difference therefore between oeso-phagostomiasis in sheep and cattle is that in the former anaemia appears to be dietetic in origin while in the latter it is parasitic.

Fourie (1936) noted an increase in eosinophile counts in sheep infested with O. columbianum, Sinclair (1962) in fascioliasis and Srivastava, Muralidharam & Dutt (1964) in schistosomiasis. This increase would appear to be due to the presence of a foreign protein, possibly some portion of the worm or its excretory products. In the present experiment eosinophilia was noted at the third to fourth week of infestation which is just prior to and at the time of the fourth moult (Veglia, 1923).

## Anthelmintic

In an anthelmintic trial Reinecke *et al.* (1963) reported the inability of thiabendazole, used at a dosage rate of 100 mg/Kg, to remove all two-day old *O. columbianum* larvae. The intestinal lesions in the sheep treated with thiabendazole in their trial were less severe, however, than in the untreated controls (Horak, 1963). Similar results were also obtained by Southcott (1963).

From these observations it appeared that, at a dosage rate of 100 mg/Kg two days after infestation, thiabendazole was able to suppress the acute effects of oesophagostomiasis but nevertheless allowed a large number of worms to mature. These suppositions were borne out in the present experiments where two of the three infested sheep not treated with thiabendazole succumbed to oesophagostomiasis (sheep 2 3 and 12). The other sheep, infested and then treated, survived the acute disease and developed chronic oesophagostomiasis.

The efficacy of 75.4 per cent against two-day old worms reported by Reinecke *et al.* (1963) was not obtained in the present trials, where there was virtually no difference in the number of worms recovered from the untreated and thiabendazole treated sheep (Table 6).

#### Pathogenicity

When compared with paramphistomiasis and ostertagiasis (Horak & Clark, 1963, 1964; Horak, Clark & Botha, 1965) relatively small numbers of worms produce severe lesions and death. It would, however, appear that secondary complications and bacterial infections as a result of larval migration play an important part in this disease.

Reinecke (1964), Rossiter (1964) and Viljoen (1964) showed that infective *O. columbianum* larvae are picked up from the grazing from February to July. These larvae cause anorexia, weight loss and diarrhoea and are themselves excreted by this diarrhoea. From July onwards fewer larvae are picked up and diarrhoea is less severe, allowing some worms to develop to adults. Anorexia continues and this plus the poor winter grazing at this time of the year, result in sheep dying of starvation in spring while harbouring only a few adult *O. columbianum*.

A diagnosis of oesophagostomiasis in such cases must rely more on the pathological changes present than on the number of worms recovered.

# SUMMARY

Trials are described in which the reactions of nine sheep infested with *O. columbianum* and three uninfested controls were studied in detail.

The main pathological findings were:-

- (i) anorexia, loss in body weight, diarrhoea and increased rectal temperature followed by subsequent reduced weight gain;
- (ii) a decrease in plasma albumin and albumin/globulin ratio and an increase in plasma gamma globulin particularly after reinfestation;
- (iii) eosinophilia commencing three to four weeks after infestation and
- (iv) a decrease in packed cell volume, haemoglobin and total plasma protein concentration, plasma volume, absolute volume of circulating erythrocytes and total circulating plasma proteins appeared to be dietetic in origin.

Marked macroscopic parasitic lesions with secondary bacterial complications were observed in all the sheep.

Infestations with comparatively few larvae led to severe disease and even death.

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