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THE PATHOLOGICAL PHYSIOLOGY OF HELMINTH INFESTATIONS. III. *TRICHOSTRONGYLUS COLUBRIFORMIS*

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INTRODUCTION

The effects of *Trichostrongylus* spp. infestation on sheep and goats have been described by Andrews (1939), Andrews, Kauffman & Davis (1944), Franklin, Gordon & MacGregor (1946), Kates & Turner (1953), Gibson (1954a, b; 1955a, b), Gallagher (1963), Gordon (1964) and Fitzsimmons (1966).

Their findings may be combined and summarized: Acute infestations result in anorexia, haemo-concentration, hypoproteinaemia, a decrease in plasma calcium and death within 28 days. Chronic infestations cause occasional diarrhoea, inappetence, weight loss, emaciation, decreased protein digestion, poor utilization of calcium and phosphates, anaemia, hypoproteinaemia, hypo-albuminaemia and death many months after initial infestation.

The present study was undertaken in an attempt to determine all the above factors in a single series of experiments and thus obtain a complete picture of the disease.

MATERIALS AND METHODS

Merino and Dorper (Dorset Horn × Black Head Persian) sheep, bred and reared worm-free were used throughout.†

Twelve sheep were divided into pairs and one member of each pair was infested with a single dose of 250,000 infective larvae of *Trichostrongylus colubriformis* and developed acute trichostrongyliasis. A further 10 sheep were also divided into pairs and one member of each pair was infested with a single dose of 100,000 larvae of *T. colubriformis* and developed chronic trichostrongyliasis.

The sheep were housed separately and lucerne hay and water were offered *ad lib.* to the infested sheep. The uninfested control sheep of each pair, which will be referred to as " feed controls ", received an amount of lucerne hay equal to that eaten by their infested partners the previous day (Bremner, 1961). Whatever other treatment the infested sheep received, similar treatment was given to their respective feed controls.

Feed and water consumption and rectal temperature were determined daily. Faecal worm egg counts were made one to four times weekly and body weight obtained weekly.

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† Despite these precautions two Dorpers were found to be lightly infested with *Skrjabinema* spp. at autopsy. The source of this infestation is unknown

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The daily nitrogen and phosphate balances of two of the chronically infested sheep and their feed controls were determined.

Two sheep which developed acute trichostrongyliasis, together with their feed controls, were each dosed 100 glass beads daily for 13 days from the eighth day of infestation onwards. Total daily faecal collections were made and the beads were recovered from the faeces by sieving on a 10 mesh to the linear inch sieve and the number excreted daily counted.

One of the sheep with acute trichostrongyliasis, and its control, were treated with thiabendazole* at 100 mg/Kg live weight at the height of the disease. Two of the chronically infested sheep were treated during the terminal phase of the disease; one and its control, with sodium selenite in aqueous solution and the other and its control, with thiabendazole at 100 mg/Kg live weight.

Blood analyses were done as described by Horak & Clark (1964).

At death or slaughter of any of the infested sheep their respective feed controls were also slaughtered so that comparisons could be made *post mortem*. The internal organs of some of the infested and control sheep were weighed and the weights compared.

Recovery and counting of worms were carried out as described by Reinecke, Snijders & Horak (1962). A 300 mesh to the linear inch sieve was used for worm recovery; the gut was not digested and three one fortieth or one fiftieth aliquots were examined for worms.

Experimental Animals

Experiment 1

Acute disease: Six sheep.

Merino 1: Worm-free feed control of Merino 2.
Merino 2: Infested with 250,000 larvae. Died 17 days later.
Dorpers 3 and 5: Worm-free feed controls of Dorpers 4 and 6 respectively.
Dorpers 4 and 6: Infested with 250,000 larvae each. Died 16 and 17 days later respectively.

Experiment 2

Acute disease: The passage of glass beads. Six sheep.

Dorpers 9 to 12 were each dosed 100 glass beads daily for 13 days from the eighth day of infestation onwards.

Merino 7: Worm-free feed control of Merino 8.
Merino 8: Infested with 250,000 larvae. Died 16 days later.
Dorper 9: Worm-free feed control of Dorper 10. When Dorper 10 died Dorper 9 became, with Dorper 11, the feed control of Dorper 12.
Dorper 10: Infested with 250,000 larvae. Slaughtered *in extremis* 16 days later.
Dorper 11: Worm-free feed control of Dorper 12.
Dorper 12: Infested with 250,000 larvae. Dosed with thiabendazole at 100 mg/Kg live weight 16 days after infestation.

* Thibenzole, registered trade mark of Merck & Co., Inc., Rahway, N.J., U.S.A.

Experiment 3

Chronic disease: Six sheep.

Merino 13: Worm-free feed control of Merino 14.

Merino 14: Infested with 100,000 larvae. Slaughtered 110 days later.

Dorper 15: Worm-free feed control of Dorper 16.

Dorper 16: Infested with 100,000 larvae. Dosed 5 mg sodium selenite *per os* 101 days later and slaughtered nine days afterwards.

Dorper 17: Worm-free feed control of Dorper 18.

Dorper 18: Infested with 100,000 larvae. Died 99 days later.

Experiment 4

Chronic disease: Nitrogen and phosphate balance determinations. Four sheep.

Dorper 19: Worm-free feed control of Dorper 20.

Dorper 20: Infested with 100,000 larvae. Died 55 days later. Nitrogen and phosphate balance determined until death.

Dorper 21: Worm-free feed control of Dorper 22.

Dorper 22: Infested with 100,000 larvae. Nitrogen and phosphate balance determined until 59 days after infestation. Treated with thiabendazole at 100 mg/Kg live weight 77 days after infestation and died the following day.

All feed controls have been given odd numbers and their infested partners have the following even numbers.

Results

Symptoms

(1) Acute disease

The sheep became listless within 13 days of infestation and a slight oedematous swelling of the muzzle and submandibular space developed. A yellow foetid, fluid diarrhoea commenced at the same time and persisted until death.

(2) Chronic disease

All the Dorpers developed a submandibular oedematous swelling 24 to 26 days after infestation, this disappeared two to eight days later but reappeared in Dorper 18 in the terminal stages of the disease.

No true diarrhoea developed but the faeces became putty-like and occasionally more fluid from the 24th to 45th day of infestation onwards. This persisted until death or slaughter.

In the terminal stages of the disease the Dorpers were emaciated, listless extremely weak and the mucous membranes became pale.

The only symptoms shown by the Merino were putty-like faeces and slightly pale mucous membranes.

Food intake and body weight

(1) Acute disease

The average daily feed intake and body weights (expressed as a percentage of the body weight before infestation) of the infested and feed control sheep in Experiment 1 are shown in Fig. 1, while the same data for Merino 8 and Dorper 10 and their feed controls in Experiment 2 are illustrated in Fig. 2. The observations for Dorper 12 and its control are illustrated in Fig. 8.

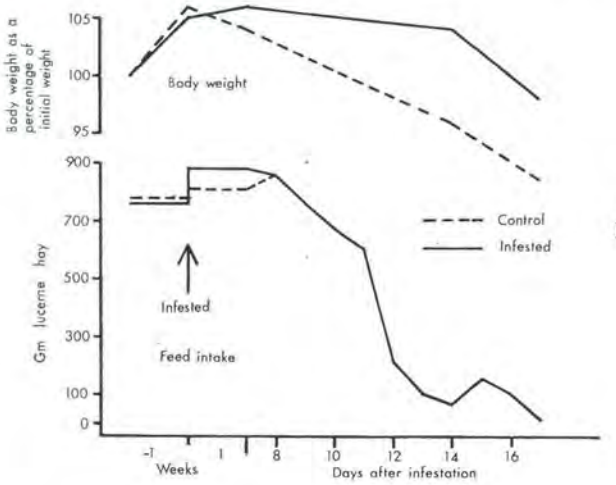

FIG. 1.—*Experiment* 1: Body weight and feed intake

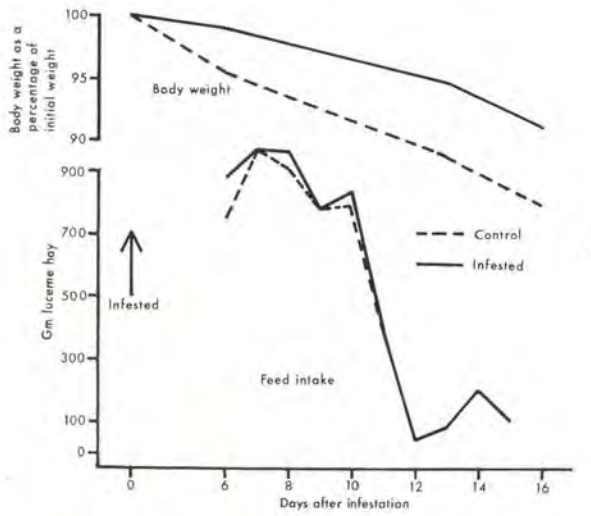

FIG. 2.—*Experiment* 2: Body weight and feed intake

Feed intake decreased markedly from the 10th day of infestation and approached complete anorexia from the 12th day. Deaths occurred on the 16th and 17th days.

In both experiments the body weight loss of feed controls was considerably greater than that of their infested partners, despite their receiving identical amounts of lucerne hay.

(2) Chronic disease

The average daily feed intake and body weights (expressed as a percentage of the body weight before infestation) of the sheep in Experiment 3 are illustrated in Fig. 3.

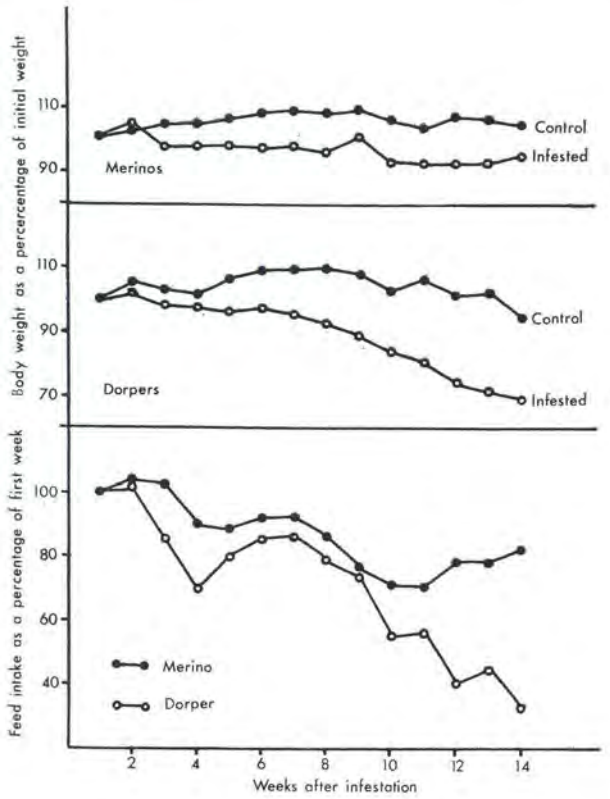

FIG. 3.—*Experiment* 3: Body weight and feed intake

The feed intake of the Dorpers was depresssed more than that of the Merino and anorexia became progressively more severe. In the Merino, feed intake improved spontaneously from the 11th week of infestation onwards. Water intake closely followed feed intake in both the infested and control sheep.

The control Merino gained a little weight while its infested partner, after losing weight initially, maintained its body weight at a slightly lower level.

A progressive divergence of body weight occurred between the Dorpers and their feed controls so that at the termination of the experiment the controls were 95 per cent of their original body weight while the infested sheep were only 70 per cent. This occurred despite the feed intake of the infested and control sheep being maintained at an identical level.

Nitrogen and phosphate intake and balance

Chronic disease

The infested sheep in Experiment 4 were offered lucerne hay and teff hay *ad lib.* The respective amounts which they consumed of each were fed to their feed controls the following day. All four sheep were kept in metabolism cages.

(1) Nitrogen

The body weights, nitrogen intake and nitrogen balance of the four sheep are shown in Fig. 4 and 5.

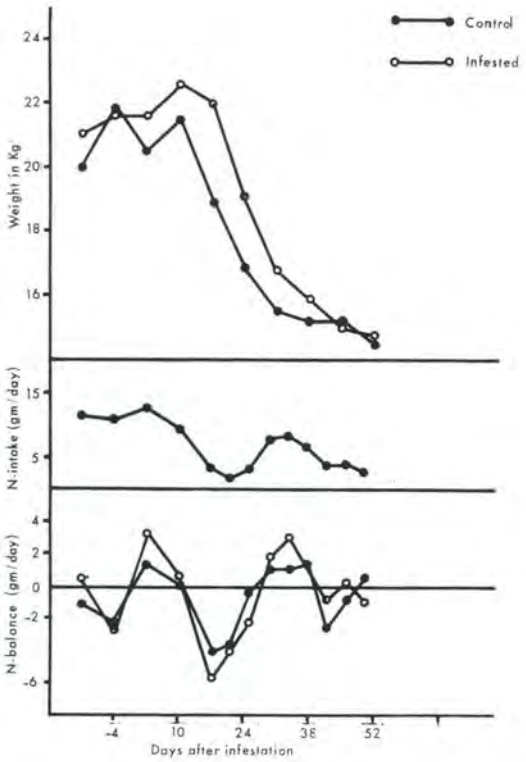

FIG. 4.—*Experiment* 4: Body weight, nitrogen intake and nitrogen balance, Dorpers 19 and 20

From the 17th day of infestation onwards a progressive weight loss in both the infested and control sheep occurred, the infested sheep finally losing approximately one kilogram more weight each than their controls.

The nitrogen intake of Dorpers 19 and 20 (Fig. 4) declined markedly after the 10th day of infestation, corresponding to the period of anorexia. It increased slightly only to drop again progressively as anorexia again became evident.

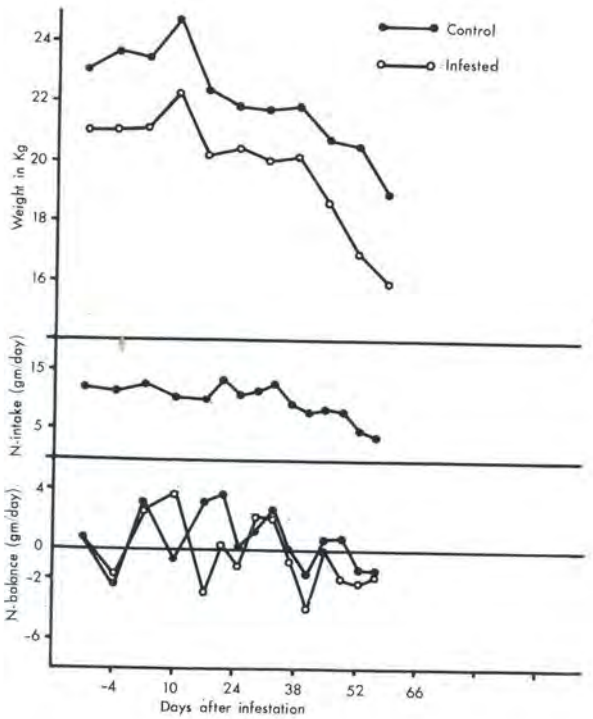

FIG. 5.—*Experiment* 4: Body weight, nitrogen intake and nitrogen balance, Dorpers 21 and 22

Nitrogen balance followed a similar trend, both sheep going into negative balance during the period of low nitrogen intake from the 10th to the 27th day of infestation and again from the 40th day of infestation. The infested sheep exhibited a greater negative balance than the control during the initial period of anorexia but during the final period the trend was less clear.

The nitrogen intake of Dorpers 21 and 22 (Fig. 5) only showed a definite progressive decrease after the 33rd day of infestation. The infested sheep (Dorper 22), however, went into negative nitrogen balance on the 17th day and thereafter, with but one exception, maintained a lower nitrogen balance than its feed control, remaining in negative nitrogen balance from the 36th day of infestation onwards.

(2) Phosphate

The phosphate intake and phosphate balance of the four sheep are illustrated in Fig. 6 and 7.

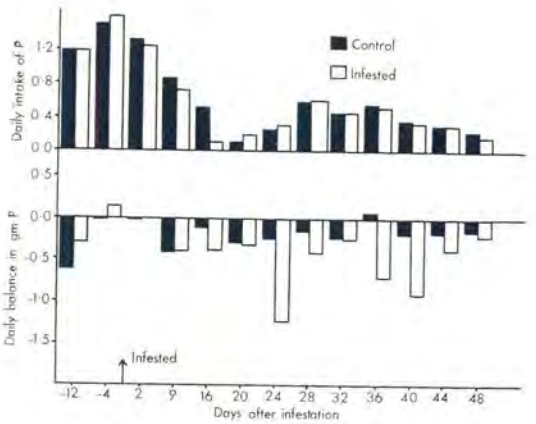

FIG. 6.—*Experiment* 4: Daily phosphate intake and balance, Dorpers 19 and 20. Figures are based on collection periods of four days

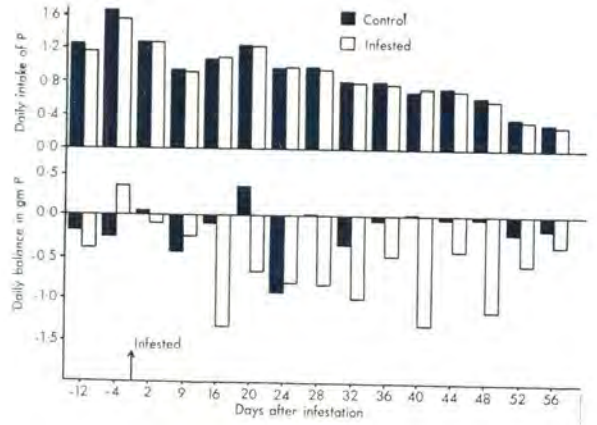

FIG. 7.—*Experiment* 4: Daily phosphate intake and balance, Dorpers 21 and 22. Figures are based on collection periods of four days

The daily phosphate intake of the infested sheep and their respective controls was similar and followed the same pattern as the nitrogen intake and thus feed intake.

Prior to and just after infestation the sheep were very nearly in complete phosphate balance but thereafter the infested sheep went into a negative balance sometimes exceeding one gram of phosphate a day. The controls although also exhibiting a slight negative balance more nearly maintained phosphate balance despite the decreased phosphate intake.

Anthelmintic treatment

(1) Acute disease

Dorper 12 and its feed control Dorper 11 were treated with thiabendazole at 100 mg/Kg live weight at the height of infestation. Anorexia was virtually complete and diarrhoea severe at the time.

The effect of this treatment on body weight and feed intake is illustrated in Fig. 8.

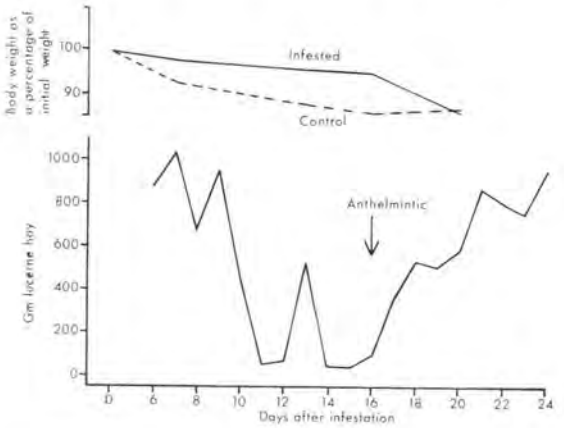

FIG. 8.—*Experiment* 2: The effect of anthelmintic treatment on the body-weight and feed intake of an acutely infested sheep

Feed intake improved immediately after treatment and was almost back to normal five days later. At the same time the body weight of the infested sheep dropped while that of the feed control remained constant. Diarrhoea ceased within 24 hours and normal faecal pellets were passed within 48 hours.

No attempt was made to determine the anthelmintic efficacy of the drug by counting excreted worms.

(2) Chronic disease

Dorper 22 was treated with thiabendazole at 100 mg/Kg, 77 days after infestation but died the following day.

The drug was apparently effective as all but a few of the worms were recovered from the caecum and colon, but the disease had progressed too far for the sheep to recover.

Sodium selenite administration

Chronic disease

The daily feed consumption of Dorper 16 and its feed control Dorper 15 before and after the administration of sodium selenite is illustrated in Fig. 9.

Within 48 hours after treatment, feed intake had increased markedly and continued increasing until the sheep was slaughtered. The average daily intake during the week preceding treatment was 318 gm, while that after dosing was 467 gm.

This treatment did not result in the expulsion of worms as the worm burden was similar to that of the other chronically infested Dorpers (Table 8).

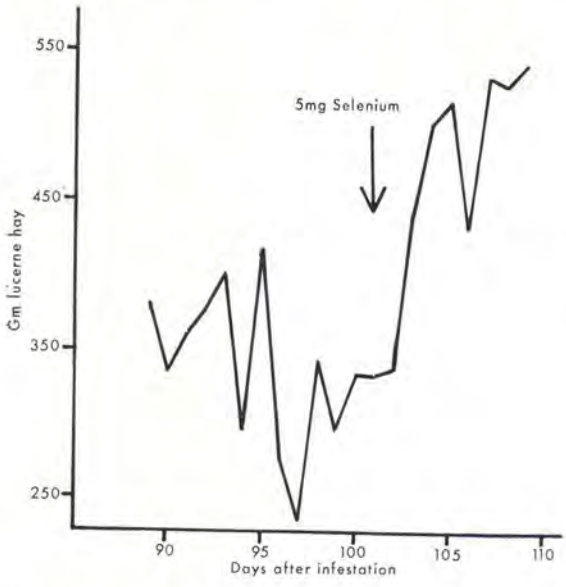

FIG. 9.—*Experiment* 3: The effect of selenium administration on the feed intake of a chronically infested sheep

The passage of glass beads

Acute disease

Dorpers 9 to 12 were each dosed 100 glass beads daily from the eighth day of infestation. The numbers of these beads recovered daily from their faeces are shown in Fig. 10.

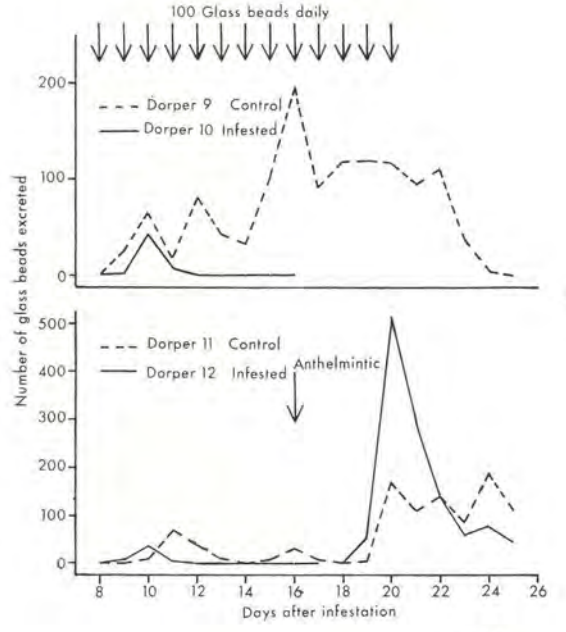

FIG. 10.—*Experiment* 2: The passage of glass beads

The infested Dorpers 10 and 12 excreted a few beads after initial dosage but thereafter no beads were excreted until Dorper 10 was slaughtered *in extremis* on the 16th day after infestation and Dorper 12 was treated with thiabendazole. Four days after this anthelmintic treatment Dorper 12 excreted 512 beads in a single day and lesser numbers thereafter.

Dorper 9, which served as a feed control for Dorper 10 and after its later death for Dorper 12, excreted beads regularly with a peak of 196 beads 16 days after infestation when feed intake was at its lowest. Dorper 11 only excreted small numbers of beads daily until its infested partner (Dorper 12) was treated with thiabendazole and its feed intake increased. Thereafter quite large numbers were excreted daily with the increased feed allowance.

At slaughter, the distribution of the glass beads in the gastro-intestinal tract of Dorper 10 was noted. At that stage this sheep had received 700 beads, only 54 of which had been excreted. Of the remaining 646 beads, 229 were in the rumen, 190 in the abomasum and 227 in the small and large intestines. This indicated a marked retention of beads in the rumen and abomasum.

Rectal temperature

There was no difference between the rectal temperatures of the infested sheep and their feed controls in the acute disease.

Chronic disease

The average weekly rectal temperatures of the sheep in Experiments 3 and 4 are shown in Fig. 11.

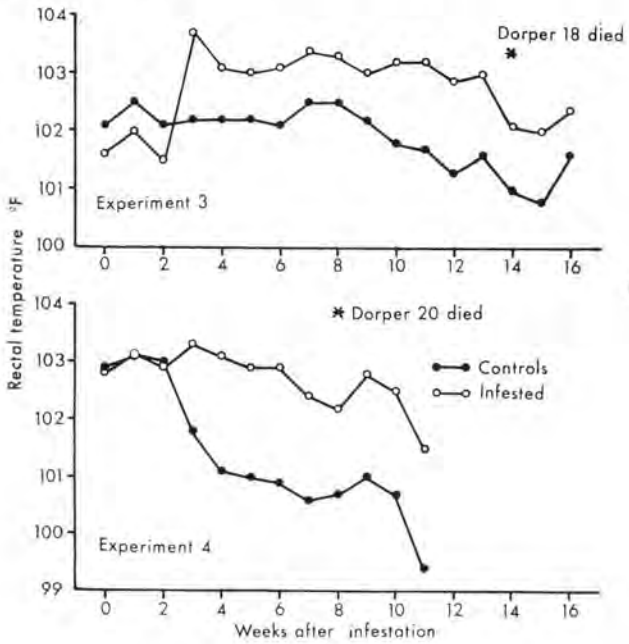

FIG. 11.—*Experiments* 3 *and* 4: Rectal temperatures of infested Dorpers and their controls

The rectal temperature of Merino 14 was no different from that of its feed control and is therefore not illustrated in Fig. 11.

The rectal temperatures of the infested Dorpers rose to a peak during the third week of infestation and thereafter remained approximately 1°F higher than that of the feed controls. The rectal temperatures of the sheep in Experiment 4 followed the same trend, although the initial temperatures of these sheep were considerably higher than those in Experiment 3.

Clinical Chemistry

(1) Acute disease

The clinical chemistry findings of the sheep in Experiment 1 are presented in Table 1 and Fig. 12 and 13.

TABLE 1.—Experiment 1.—*The clinical chemistry findings of acutely infested sheep*

Days after infestation		−6	+1	+8	+15	+17
Packed cell volume %	*M1	32	29	26	27	
	M2	29	29	27	34	
	**D3	31	32	28	31	
	D4	36	34	35	48	
	@D5	33	33	31	32	29
	D6	34	35	32	34	35
Haemoglobin gm%	M1	11·9	11·1	10·0	10·5	
	M2	11·6	11·1	10·3	13·5	
	D3	12·7	12·7	11·1	13·0	
	D4	14·3	13·5	13·2	19·2	
	D5	13·0	12·7	11·9	13·0	11·6
	D6	13·2	13·2	12·4	13·5	12·4
Plasma calcium mg %	M1	12·3	12·3	12·3	9·4	
	M2	12·9	14·1	12·3	7·9	
	D3	12·9	12·9	12·9	12·1	
	D4	12·3	12·9	14·1	7·6	
	D5	12·9	12·9	13·5	10·5	10·0
	D6	12·9	12·3	12·9	7·9	8·4
Plasma inorganic phosphate mg %	M1	7·3	4·6	6·9	8·6	
	M2	7·6	3·8	2·8	5·5	
	D3	8·5	5·6	8·0	7·0	
	D4	6·3	4·0	4·7	6·0	
	D5	6·6	4·2	7·7	10·8	10·6
	D6	6·3	4·3	3·7	6·2	4·9
Blood sugar mg %	M1	93	74	63	56	
	M2	67	63	47	52	
	D3	71	71	64	75	
	D4	73	70	62	59	
	D5	75	73	71	56	58
	D6	92	81	66	52	62
Eosinophiles/cub mm	M1	75	65	65	65	
	M2	65	50	25	0	
	D3	150	140	125	175	
	D4	65	50	50	0	
	D5	25	20	15	0	
	D6	225	175	40	0	

*M = Merino **D = Dorper @Odd numbers are controls, even numbers infested sheep

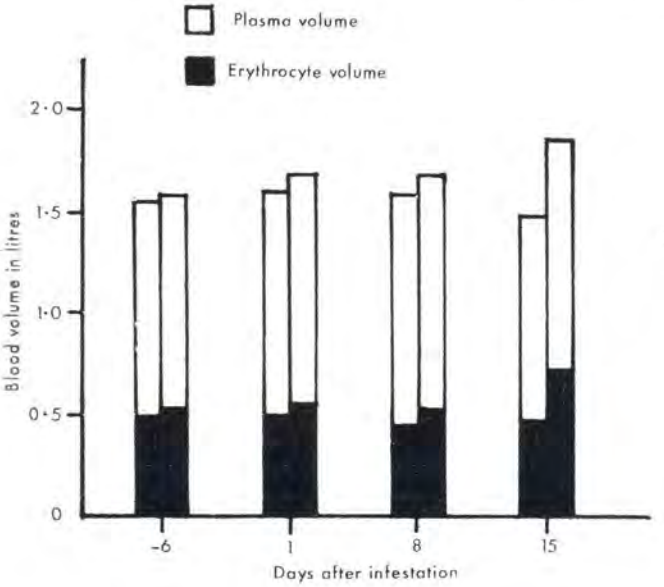

FIG. 12.—*Experiment* 1: The average blood volumes of infested and control sheep. The readings for the controls are given on the left

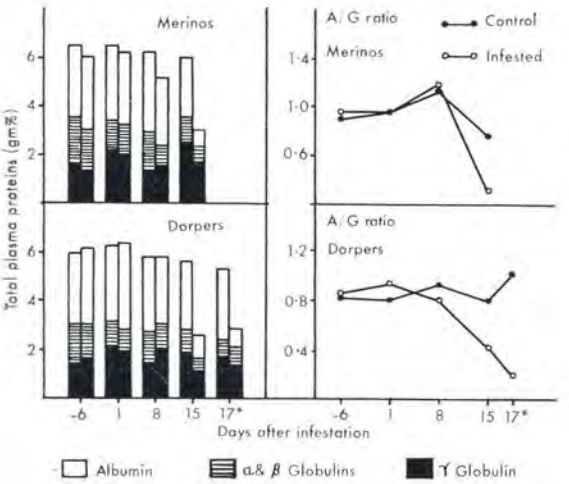

FIG. 13.—*Experiment* 1: Plasma protein fractions. The readings for the controls are given on the left. A/G ratio = Albumin/Globulin ratio.
* Readings for Dorpers 5 and 6 only as the other infested Dorper had died

The infested sheep showed an increase in packed red cell volume, haemoglobin concentration and total volume of circulating erythrocytes just prior to death (Table 1 and Fig. 12).

Fifteen days after infestation the three infested sheep had low plasma calcium concentrations; plasma inorganic phosphate concentrations within normal limits, but lower than those of the feed controls; normal blood sugar concentrations and zero eosinophile counts (Table 1).

In both the infested Merino and the infested Dorpers a very severe drop in total plasma protein concentration and albumin/globulin ratio, almost entirely due to a drop in plasma albumin concentration, was evident 15 days after infestation (Fig. 13).

(2) The effect of anthelmintic treatment on the acute disease

The clinical chemistry findings of three Dorpers (Dorpers 10, 11 and 12) in Experiment 2, prior to and after anthelmintic treatment are given in Table 2.

TABLE 2.—EXPERIMENT 2.—*The effect of anthelmintic treatment on the clinical chemistry of the acute disease*

Days after Infestation	Sheep No.	Packed Cell Volume	Haemoglobin	Total Plasma Proteins	Plasma Volume	Circulating Red Blood Cell Volume	Plasma Inorganic Phosphate
		%	gm%	gm%	(litre)	(litre)	mg%
16	*D10	42	17·3	3·86	1·36	0·98	7·1
	@D11	35	13·5	7·20	1·27	0·68	6·6
	D12	45	18·6	3·70	1·04	0·85	4·7
16	Dorper 10 slaughtered *in extremis*. Dorpers 11 and 12 treated with thiabendazole at 100 mg/Kg						
22	D11	33	12·7	6·82	1·23	0·60	5·9
	D12	29	11·1	6·32	1·23	0·50	3·3

* D = Dorper @ Odd numbers are control sheep, even numbers infested sheep

The packed red cell volumes, haemoglobin concentrations and total volumes of circulating erythrocytes of the two infested sheep (Dorpers 10 and 12) were considerably higher, while their total plasma protein concentrations were lower than those of the feed control (Dorper 11) on the day of treatment. Dorper 10 was slaughtered *in extremis* while Dorper 12 and its control were treated with thiabendazole at 100 mg/Kg live weight on the 16th day of infestation.

Six days after treatment all factors had returned to normal levels (Table 2) as had the feed intake (Fig. 8) and passage of ingesta (Fig. 10).

(3) Chronic disease

The clinical chemistry findings of the six sheep in Experiment 3 are presented in Table 3 and Fig. 14 to 17.

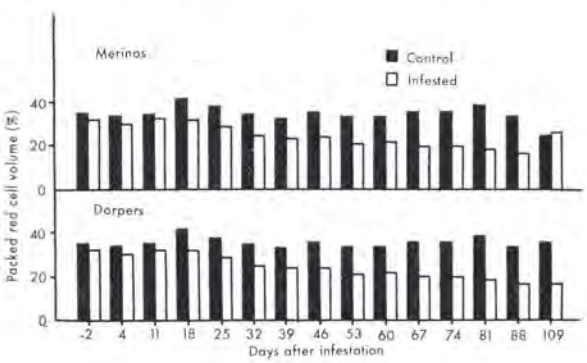

FIG. 14.—*Experiment* 3: The packed red cell volumes of chronically infested sheep

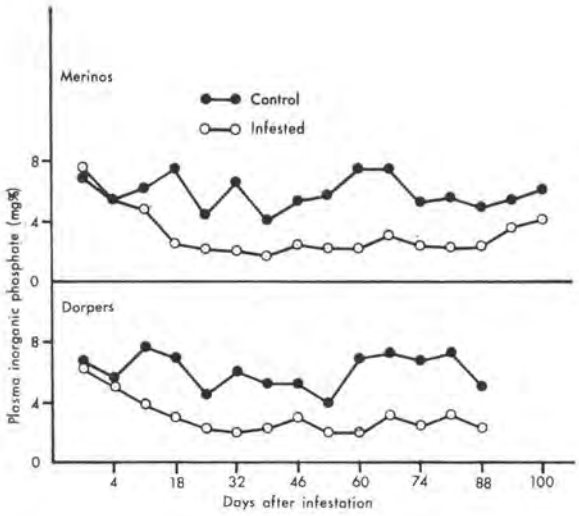

FIG. 15.—*Experiment* 3: Plasma inorganic phosphate concentrations

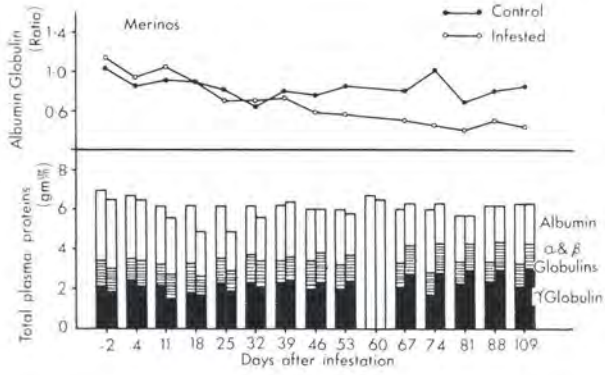

FIG. 16.—*Experiment* 3: The plasma protein fractions of the chronically infested Merino and its worm-free control. The readings for the control are given on the left

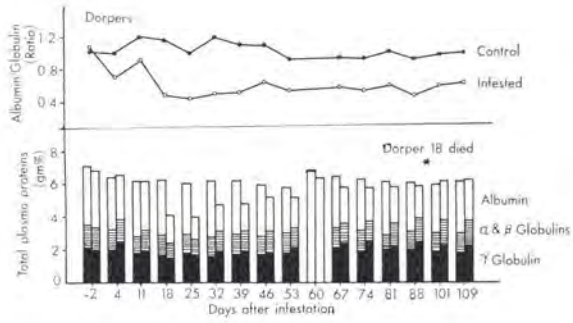

FIG. 17.—*Experiment* 3: The plasma protein fractions of the chronically infested Dorpers and their worm-free controls. The readings for the controls are given on the left

TABLE 3.—EXPERIMENT 3.—*The clinical chemistry findings of chronically infested sheep and their worm-free controls*

Week of infestation		0	2	4	6	8	10	12	13	16
Haemoglobin (gm %)	*M13	10·3	10·3	9·7	10·0	9·7	10·0	9·2	8·6	9·7
	M14	10·0	10·6	10·6	8·4	8·1	7·3	7·6	7·2	10·0
	**D15	12·4	12·2	14·1	12·1	12·2	12·2	14·6	11·6	14·3
	D16	11·1	11·6	10·6	8·6	7·8	7·6	7·0	5·7	6·8
	@D17	13·5	14·1	14·3	12·8	14·1	14·1	14·0	14·3	
	D18	11·6	12·2	11·4	9·5	8·9	8·7	8·1	8·6	
Plasma volume (litre)	M13	1·24	1·31	1·25	1·33	1·20	1·22	1·19	1·17	1·15
	M14	1·08	1·25	1·20	1·31	1·24	1·22	1·22	1·19	1·11
	D15	1·31	1·38	1·25	1·31	—	1·22	1·15	1·13	1·08
	D16	1·44	1·56	1·63	1·45	1·72	1·80	1·60	1·75	1·53
	D17	1·61	1·79	1·47	1·71	1·80	1·47	1·57	1·44	
	D18	1·47	1·38	1·56	1·68	1·47	1·47	1·44	1·50	
Circulating red blood cell volume (litre)	M13	0·39	0·48	0·44	0·45	0·40	0·45	0·38	0·37	0·38
	M14	0·34	0·46	0·44	0·41	0·33	0·28	0·33	0·30	0·39
	D15	0·67	0·68	0·73	0·59	—	0·52	0·74	0·56	0·61
	D16	0·61	0·84	0·63	0·43	0·40	0·42	0·35	0·33	0·31
	D17	0·90	1·00	0·94	0·92	1·05	1·02	0·96	0·77	
	D18	0·63	0·62	0·66	0·52	0·41	0·39	0·38	0·33	
Plasma calcium (mg %)	M13	12·4	12·3	10·5	12·3	11·8	10·5	12·2	12·2	11·8
	M14	11·6	11·8	10·5	11·2	11·8	10·0	10·5	11·1	11·8
	D15	12·0	11·8	10·5	11·2	11·8	11·1	11·1	12·2	12·3
	D16	12·0	11·8	9·4	11·2	12·3	11·1	11·4	11·6	12·9
	D17	12·4	12·3	10·5	11·7	12·3	11·1	11·1	12·2	
	D18	12·0	11·8	10·0	10·6	11·8	10·0	11·1	11·6	
Blood sugar (mg %)	M13	62	56	58	67	59	52	51	55	60
	M14	71	53	66	70	63	62	66	58	53
	D15	78	70	64	70	71	66	58	60	55
	D16	77	69	74	77	107	75	80	79	106
	D17	88	71	73	88	70	73	77	79	
	D18	78	62	64	74	62	67	66	64	
Eosinophiles per cub mm	M13	90	60	20	30	40	40	40	100	70
	M14	75	130	20	20	420	160	150	150	200
	D15	75	120	70	40	120	30	10	40	30
	D16	75	30	0	60	40	20	400	40	30
	D17	120	70	20	20	50	30	20	40	
	D18	450	120	10	40	320	30	70	80	

*M = Merino **D = Dorper @Odd numbers are controls, even numbers infested sheep

In both Merino and Dorper sheep the infestation caused a marked drop in packed red cell volume, haemoglobin concentration and total volume of circulating erythrocytes after the 26th day of infestation. At the end of the experiment the Merino was showing signs of recovery whereas the Dorpers were not (Table 3 and Fig. 14).

The changes in haemoglobin concentration correspond to those seen in the packed red cell volume and an examination of their relationships does not reveal any demonstrable changes in haemoglobin per unit volume of red cells. As red cell counts were not made the indices cannot be calculated and the exact type of anaemia present cannot be stated, but the available evidence would not indicate any marked changes in mean red cell haemoglobin content.

As the plasma volume showed only a slight rise throughout the experiment and the total volume of circulating erythrocytes parallelled the fall in packed red cell volume, the possibility of the low haematocrit values being due to haemodilution is excluded. The feed controls showed a fairly substantial decrease in plasma volume.

The infested Merino maintained a fairly high eosinophile count from the 54th day of infestation onwards whereas the Dorpers each had a single high count, the eosinophile counts at other times being similar to those of the controls (Table 3).

The plasma inorganic phosphate concentrations in the infested Merino and Dorper sheep decreased from the 12th day of infestation and reached a level of approximately 2 mg per cent on the 26th day. Thereafter there was little evidence of recovery excepting in the Merino during the terminal stages of the experiment (Fig. 15).

The infested sheep developed a marked hypoproteinaemia, mainly due to a decrease in plasma albumin concentration from the 18th to 54th day of infestation. As recovery took place the gain in total plasma protein concentration in the infested sheep was largely due to an increase in plasma gamma globulins, particularly in the Merino, thus causing a further decline in the albumin/globulin ratio (Fig. 16 and 17).

A slight decline occurred in the total plasma protein concentration of the feed controls and was probably caused by diminished feed intake.

Changes in the total circulating plasma proteins and the albumin/globulin ratio are summarized in Table 4.

TABLE 4.—EXPERIMENT 3: *The total circulating plasma proteins*

Chronically infested sheep

Protein Fractions	Initial gm	At Lowest T.C.P.P. gm	% Change	Final T.C.P.P. gm	% Change
Total circulating plasma albumin gm	47·2	24·9	−47·2	29·3	−37·1
Total circulating plasma α & β globulin gm	15·8	14·9	−5·7	18·5	+17·1
Total circulating plasma γ globulin gm	25·8	24·9	−3·5	33·8	+31·0
Total circulating plasma protein gm.	88·8	64·7	−27·1	81·6	−8·1
A/G Ratio	1·13	0·63		0·56	

Uninfested feed control sheep

Total circulating plasma albumin gm.	49·9	34·3	−31·3	36·8	−26·3
Total circulating plasma α & β globulin gm	17·4	13·7	−21·3	14·1	−19·0
Total circulating plasma γ globulin gm	27·5	23·6	−14·2	23·8	−13·5
Total circulating plasma proteins gm	94·8	71·6	−24·5	74·7	−21·2
A/G Ratio	1·11	0·92		0·97	

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The lowest total circulating plasma protein values were encountered during the acute stage of the disease and these low values were due mainly to a drop in total circulating plasma albumin in the infested sheep whereas all fractions were nearly equally affected in the controls.

The final total circulating plasma protein value showed only a slight drop from the initial value in the infested sheep. This drop was entirely due to a decrease in plasma albumin for both plasma alpha and beta globulin and gamma globulin showed a substantial increase, thus further depressing the albumin/globulin ratio.

In the feed controls, however, the final circulating plasma protein value was 20·1 gm below the initial level and this drop was due to a decrease in all fractions, the albumin/globulin ratio thus remaining fairly constant throughout the experiment.

The amount of total circulating plasma protein is dependent upon the plasma volume. In the infested sheep the average plasma volume increased from an initial value of 1·33 litres to a final value of 1·38 litres, an increase of 50 ml, while the average total plasma protein concentration decreased from 6·70 gm per cent to 6·30 gm per cent (Fig. 16 and 17), thus accounting for the small change in the total circulating plasma protein value at the end of the experiment.

In the feed controls the average plasma volume decreased by 170 ml from 1·39 litres initially to 1·22 litres finally while the total plasma protein concentration decreased from 7·07 gm per cent to 6·23 gm per cent (Fig. 16 and 17). The substantial fall in both plasma volume and total plasma protein concentration accounted for the final decrease in total circulating plasma proteins.

Plasma calcium, magnesium and copper and blood sugar concentrations were determined regularly for all the sheep during the course of Experiment 3 and showed no variations from the normal.

The clinical chemistry findings of the four sheep in Experiment 4 are given in Table 5 and illustrated in Fig. 18.

A slight decrease in packed red cell volume, haemoglobin concentration and red cell count and a more marked decrease in plasma inorganic phosphate occurred in both the infested sheep (Table 5).

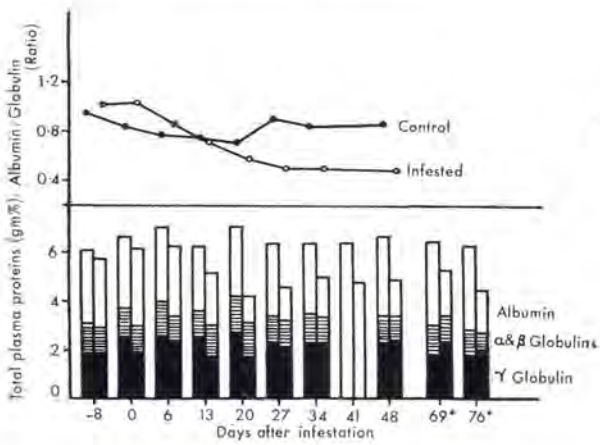

FIG. 18.—*Experiment* 4: Average values for plasma protein fractions. The values for the worm-free controls are given on the left.
* Values for Dorpers 21 and 22 only as Dorper 20 had died

TABLE 5.—EXPERIMENT 4: *Clinical chemistry findings*

Days after infestation		0	6	20	27	34	49	54	69	76
Packed cell volume %	*D19	37	39	37	42	39	38	38		
	D20	34	35	39	35	33	27	30		
	@D21	34	37	30	32	31	34	35	36	35
	D22	35	39	30	34	29	27	26	29	25
Haemoglobin (gm%)	D19	13·8	13·2	14·6	15·9	15·6	16·1	14·8		
	D20	13·8	14·6	15·1	13·2	13·5	11·5	11·3		
	D21	13·2	13·0	11·7	12·1	12·1	13·5	—	13·9	13·5
	D22	14·6	13·2	12·9	13·0	12·4	11·1	11·3	11·3	10·1
Red cell count 10^6/cub mm	D19	—	15·8	15·5	—	—	15·3	—		
	D20	—	13·1	12·0	—	—	9·7	—		
	D21	—	14·1	12·1	—	—	13·6	—	—	11·8
	D22	—	15·5	14·4	—	—	10·5	—	—	9·9
Plasma inorganic phosphate (mg%)	D19	5·4	4·1	5·6	5·9	4·9	5·7	—		
	D20	5·4	4·0	5·0	3·5	1·8	2·0	3·0		
	D21	3·7	4·2	4·9	4·2	3·6	3·3	6·6	6·0	6·3
	D22	4·2	4·6	2·7	2·0	1·9	1·7	3·6	2·1	3·3

*D = Dorper @Odd numbers are controls, even numbers infested sheep

A marked drop in total plasma protein concentration, due largely to a drop in plasma albumin occurred from the 13th day of infestation. There was little recovery in this respect during the remainder of the experiment (Fig. 18).

Faecal worm egg counts

(1) Acute disease

No eggs were recovered from the faeces of any of these sheep as they died within the prepatent period of infestation.

(2) Chronic disease

The average weekly faecal worm egg counts of the sheep in Experiment 3 are shown in Fig. 19 and those in Experiment 4 in Fig. 20. The average daily faecal output of Dorper 22 is shown in Fig. 20, that of Dorper 20 is not included because of the fluid nature of its faeces.

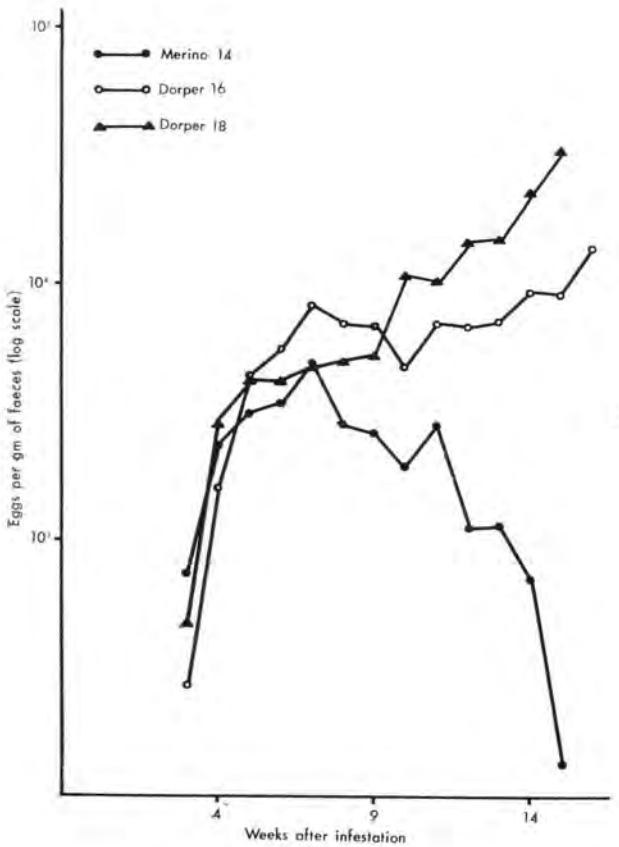

FIG. 19.—*Experiment* 3: Faecal worm egg counts

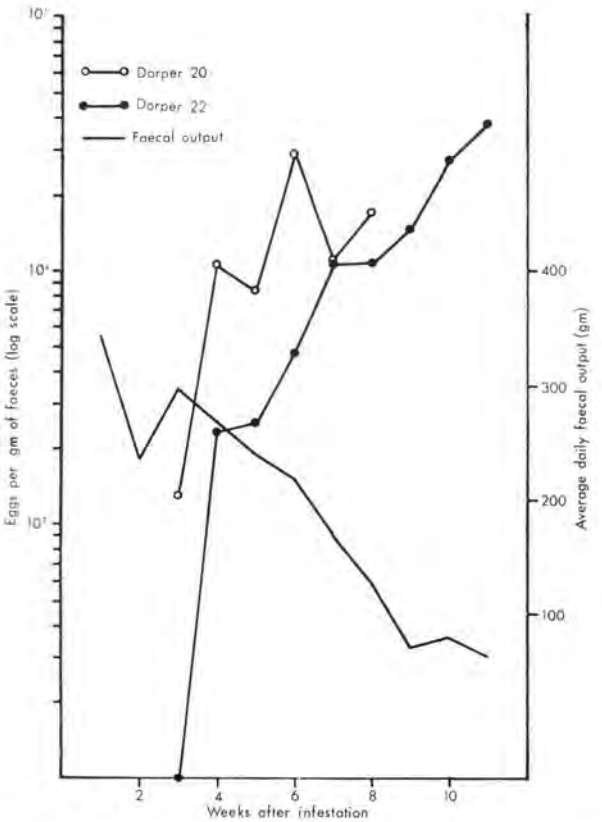

FIG. 20.—*Experiment* 4: Faecal worm egg counts and daily faecal output

The first eggs were seen in the faeces 17 and 18 days after infestation. The faecal egg counts of Merino 14 rose to a peak of 4,833 eggs per gram (e.p.g.) of faeces during the seventh week of infestation and thereafter became negative during the sixteenth week of infestation. Dorpers 16, 18 and 22 exhibited a steady climb in faecal worm egg counts and the highest counts of 13,700 e.p.g., 32,600 e.p.g. and 37,600 e.p.g. respectively were recorded in the week preceding death or slaughter. In Dorper 20 counts rose to a peak of 28,625 e.p.g. two weeks before death and declined to 17,200 e.p.g. in the week preceding death.

The average daily faecal output of Dorper 22 decreased progressively while the faecal worm egg count increased over the same period.

Pathological anatomy

The following lesions were noted:—

(1) Acute disease

Macroscopic pathology: Ruminal atony and atrophy, abomasal atony and distension, accumulation of dry ingesta in the rumen and abomasum, circumscribed raised areas in the pyloric region of the abomasum covered with green necrotic material, duodenitis, distension of the small intestine with fluid, serous atrophy of

the mesenteric fat along the caecum and colon, severe fluid diarrhoea, *tumor hepatis* and *tumor renales*, fatty degeneration of the liver and kidneys, splenic atrophy, ascites, hydro-thorax, hydro-pericardium and pulmonary oedema.

Microscopic pathology: Focal necrosis of the abomasal mucosa containing numerous branching, septate, coarse mycelia suggestive of mucormycosis, a mild, focal, round cell infiltration in the duodenal mucosa.

(2) Chronic disease

Macroscopic pathology: Emaciation, muscular atrophy, ruminal atrophy and atony, in some sheep large accumulations of dry ingesta in the rumen and abomasum and circumscribed green necrotic areas in the pyloric region of the abomasum, enteritis, *tumor hepatis* and *tumor renales* and fatty degeneration of the liver and kidneys, splenic atrophy, myocardial atrophy (in some sheep necrosis of the myocard particularly of the right ventrical and apex of the heart, the wall of the right ventricle being nearly translucent), ascites, hydro-thorax, hydro-pericardium and pulmonary oedema.

Microscopic pathology: Focal necrosis of the abomasal mucosa containing mycelia suggestive of mucormycosis, mild, focal, round cell infiltration in the duodenal mucosa, extensive fatty metamorphosis of the liver, which is most severe peripherally, in some sheep degeneration, necrosis and mineralization of the myocard with round cell infiltration and mild early fibroplasia with similar but milder changes in the skeletal musculature. Mild degeneration of the myocard and skeletal musculature in the feed controls.

Body and organ weights

The initial and final body weights and the weight of the entire intestinal tract including the rumen and contents, of those sheep in which these weights were taken, are presented in Table 6.

TABLE 6.—*Body weights and various organ weights*

Sheep No.	Initial total body weight	Final total body weight	Weight of organs in grams			
			Entire gut plus ingesta	Liver	Kidneys	Spleen
Experiment 1						
	Kg	Kg	gm	gm	gm	gm
Merino 1@	15·2	12·7	2,337	180	46	14
Merino 2	17·4	16·1	4,820	355	72	20
Dorper 5	16·5	14·5	3,580	215	45	21
Dorper 6	17·0	16·8	5,815	232	40	13
Experiment 2						
Merino 7	19·5	15·9	—	—	—	—
Merino 8	19·1	17·9	5,800	310	75	23
Dorper 9	20·6	18·1*	—	—	—	—
Dorper 10	20·4	18·1	4,625	300	58	15
Experiment 4						
Dorper 19	20·0	14·1	2,772	155	33	12
Dorper 20	21·0	14·1	3,780	385	50	10

* This weight was taken at the time Dorper 10 died
@ Odd numbers worm-free feed controls, even numbers infested sheep

In the sheep suffering from acute trichostrongyliasis the feed controls lost more weight than their infested partners, while in the chronic disease the infested sheep lost more weight than its feed control.

In all cases the weight of the entire gut of the infested sheep was considerably greater than that of their respective feed controls. The average weight of the former (including Merino 8 and Dorper 10) was 4,968 gm compared to 2,896 gm for the latter. The average difference of more than 2,000 gm compensates for the greater total body weight loss of the feed controls and accentuates the weight loss of the chronically infested sheep.

The average liver weight of the feed controls was 183 gm compared to 316 gm for that of the infested sheep, while the average kidney weights were 41 and 59 gm respectively. There was practically no difference between the average splenic weights of the infested and control sheep.

In four sheep the total weight of the abomasum, small intestine and caecum and colon plus their ingesta was obtained. The ingesta was thoroughly mixed with water, collected in bottles, allowed to sediment and the actual weight of each organ obtained. After sedimentation the volume of solid ingesta at the bottom of each bottle was measured. These results are presented in Table 7.

TABLE 7.—*Gastro-intestinal organ weights and ingesta volumes*

Sheep No.	Organ	Total weight (Organ plus ingesta)	Weight of organ	Volume of solid ingesta after sedimentation
		gm	gm	ml
Experiment 2				
Merino 8	Abomasum	645	80	340
	Intestine	820	354	180
	Caecum & Colon	470	283	15
Dorper 10	Abomasum	640	109	300
	Intestine	725	313	70
	Caecum & Colon	595	283	20
Experiment 4				
Dorper 19	Abomasum	112	58	212
	Intestine	200	160	31
	Caecum & Colon	345	163	365
Dorper 20	Abomasum	450	82	420
	Intestine	725	274	91
	Caecum & Colon	685	212	124

The total weights of each respective organ (plus ingesta) in the infested sheep (Merino 8, Dorpers 10 and 20) were considerably heavier than the corresponding weights in the one feed control (Dorper 19). The actual organ weights of the infested sheep were also greater than those of the feed control, particularly the small and large intestinal weights.

In the feed control the greatest volume of solid, sedimented ingesta was recovered from the caecum and colon. The largest volume of ingesta was obtained from the abomasa of the infested sheep while very little solid ingesta was recovered from the small or large intestines.

Worms recovered post mortem

Results are summarized in Table 8.

TABLE 8.—*The numbers of* T. colubriformis *recovered at autopsy*

Sheep No.	No. of larvae dosed	Age of infestation in days	Stage of development		
			5th	Adult	Total
Experiment 1					
Merino 2*	250,000	17	67,852	31,931	99,783
Dorper 4*	250,000	16	105,719	33,385	139,104
Dorper 6*	250,000	17	22,634	64,419	87,053
Experiment 2					
Merino 8*	250,000	16	73,697	76,703	150,400
Dorper 10*	250,000	16	45,922	56,128	102,050
Experiment 3					
Merino 14	100,000	110	0	21,293	21,293
Dorper 16	100,000	110	0	60,340	60,340**
Dorper 18*	100,000	99	0	59,880	59,880***
Experiment 4					
Dorper 20*	100,000	55	2,601	62,408	65,009
Dorper 22*	100,000	78	0	49,900	49,900

* Died or slaughtered *in extremis*

** 100 *Skrjabinema* spp. recovered

*** 1 *Skrjabinema* sp. recovered

(1) Acute disease

Considerable variations occurred in the numbers of worms recovered from the sheep which died from acute trichostrongyliasis (87,053 to 150,400) and many of these worms were still in the fifth stage. There was little difference between the worm burdens of the Merinos and Dorpers. The numbers recovered from the abomasa of the various sheep varied between 150 and 6,540 and those from the large intestine between 1,300 and 12,947. The remainder was recovered from the small intestine.

(2) Chronic disease

The worm burdens of the Dorpers were remarkably similar, three of these varying between 59,880 and 65,009. The fourth (Dorper 22) was treated with thiabendazole at 100 mg/Kg live weight on the previous day and harboured 49,900 worms; with the exception of 350 they were all present in the caecum and colon. In the other Dorpers nil to 25 worms were present in the abomasum and 60 to 2,917 in the large intestine; the remainder was recovered from the small intestine. With the exception of those in one sheep (Dorper 20), which had 2,601 fifth stage worms, all the worms were adult. Dorpers 16 and 18 were also infested with 100 and with one *Skrjabinema* spp. respectively. No other species were present.

The Merino had a worm burden considerably lower than that of any of the Dorpers and the adult female worms in this sheep contained very few or no eggs.

Discussion

Acute disease

(1) Clinical observations

The complete anorexia which develops in acute trichostrongyliasis closely resembles that reported in acute paramphistomiasis (Horak & Clark, 1963) and acute oesophagostomiasis (Horak & Clark, 1966). Unlike either of these diseases in which the infested sheep lost weight rapidly, the sheep infested with *T. colubriformis* maintained their body weight while the feed controls lost weight. This apparent maintenance of total body weight was almost entirely dependent upon the amount of ingesta present in the gastro-intestinal tract, and was shown by the large amount of solid ingesta retained in the rumen and abomasum of the infested sheep when compared with their feed controls. Further evidence of this retention was seen in the delayed passage of glass beads by the infested sheep. Fluid, however, passed the abomasal sphincter freely as shown by the fluid nature of the intestinal contents. The retention of solid ingesta was probably as a result of reflex closure of the pyloric sphincter caused by pain in the small intestine due to the massive infestation.

Anthelmintic treatment during this phase of the disease removed the worms, the cause of the feed retention, allowing passage of the glass beads. Because the retained ingesta was rapidly excreted the infested sheep lost weight immediately after anthelmintic treatment.

The rapid recovery of the sheep after treatment is confirmation of the efficacy of the anthelmintic and the remarkable recuperative powers of the small intestine. Similar observations were made in sheep heavily infested with *Paramphistomum microbothrium* and treated at the height of the infestation with *N-(2/ chlor-4-nitrophenyl)-5-chlorsalicylamid at 50 mg/Kg live weight (Horak & Clark, 1963).

(2) Clinical chemistry

A rise in packed red cell volume and haemoglobin concentration is a finding common to numerous helminth infestations. It occurs during the acute phase of the disease approximately two weeks after infestation and has been recorded in ostertagiasis (Todd, Arbogast, Wyant, Stone & Elam, 1951; Horak & Clark, 1964), paramphistomiasis (Horak & Clark, 1963), oesophagostomiasis (Horak & Clark, 1966) and trichostrongyliasis (Leland, Drudge & Wyant, 1959; Fitzsimmons, 1966). This rise is not due to a shrinkage of plasma volume as an actual increase in the volume of circulating erythrocytes takes place. The reason for this is unknown.

The decrease in total plasma protein concentration, particularly plasma albumin, is similar to that in goats acutely infested with *T. colubriformis* (Fitzsimmons, 1966) and closely resembles that which occurs in acute paramphistomiasis (Horak & Clark, 1963). Fitzsimmons (1966) reported patchy superficial necrosis of the mucosa of the small intestine of infested goats and it is presumably through these lesions that the plasma albumin is lost by seepage.

* Lintex, Farbenfabriken Bayer A.G., Leverkusen

The remarkable recovery of the plasma protein concentration of Dorper 12 (Table 2) six days after anthelmintic medication is contrary to the findings in acute paramphistomiasis (Horak & Clark, 1963) in which the total plasma protein concentration was restored 17 to 31 days after treatment. The plasma protein fractions involved in this recovery were not determined.

The decrease of plasma calcium concentration was probably due to a loss of albumin-bound calcium through seepage into the intestine.

(3) Pathological anatomy

The fatty changes observed in the liver and the kidneys are suggestive of the changes associated with ovine ketosis and could have been precipitated by the sudden onset of complete anorexia. The high blood sugar levels of these sheep are not typical of ketosis, but had the sheep survived the infestation for a longer period hypoglycaemia may have occurred.

The fungal infection of the abomasal mucosa was probably secondary to the abomasal stasis and feed retention.

The combined effects of starvation, liver involvement, circulatory failure and pulmonary oedema resulted in death.

Chronic Disease

(1) Clinical observations

The onset of anorexia in chronic trichostrongyliasis is insidious, the sheep consuming progressively less feed as the infestation progresses. Decreased feed intake is accompanied by an interference with the assimilation of certain elements, proteins and phosphates being the most important, while selenium uptake may also be reduced. The common name " bankrupt worm " is particularly apt when applied to *T. colubriformis* for although infested sheep consume feed they derive little benefit from it and the stockowner unknowingly is feeding uneconomic units.

The reduced assimilation of feed accounts for the difference in body weights between infested animals and their worm-free controls on the same intake. Similar observations have been made on sheep infested with *T. colubriformis* (Franklin, Gordon & MacGregor, 1946) and with *T. axei* (Gibson, 1955b).

The apparent interference with selenium uptake is of considerable importance in certain areas of South Africa where the available grazing or feed is already low in selenium content. The lucerne hay fed to the sheep in these experiments was low in selenium content and all the sheep had received two to three oral doses of three to five milligrams of sodium selenite prior to the commencement of the experiments. This apparently was sufficient to prevent symptoms or frank lesions of white muscle disease developing in the feed controls despite their reduced intake of the already deficient lucerne hay. Amongst the infested sheep, however, Dorper 18 developed a submandibular oedema in the terminal stages of the disease which at autopsy proved to be due to the severe atrophy, degeneration and necrosis of the myocard. Similar lesions were present in most of the other infested sheep. In a goat chronically infested with *T. colubriformis*, Fitzsimmons (1966) noted considerable myocardial degeneration, but did not suggest a possible cause of the lesion.

The dramatic improvement in feed intake by Dorper 16 after selenium administration without any apparent diminution of worm burden further supports the contention that *T. colubriformis* infestation can produce hyposeleniosis.

In the light of these observations the treatment of sheep with chronic trichostrongyliasis could consist of the administration of an effective anthelmintic followed by three to five milligrams of sodium selenite *per os*.

(2) Clinical chemistry

Lapage (1962) states that *Trichostrongylus* spp. are blood-suckers, yet the mouthparts of the worms are not adapted to blood-sucking and it seems unlikely that the anaemia encountered in chronic trichostrongyliasis is produced in this way. A more acceptable theory is that the anaemia is aplastic in origin due to a toxin excreted or produced by the parasite, or due to interference with the uptake of substances necessary for the formation of red blood cells. If this is so it corresponds to the anaemia in ostertagiasis which is not haemorrhagic or haemolytic or produced by bloodsucking (Horak, Clark & Botha, 1965).

The anaemia of trichostrongyliasis or ostertagiasis is not as severe as that produced by the bloodsucking nematodes *Haemonchus contortus* or *Gaigeria pachyscelis*, but it underlines the common occurrence of this symptom in helminth infestations. The cause of this anaemia, particularly in infestations with non-bloodsucking worms, must still be determined.

Another similarity between trichostrongyliasis and ostertagiasis is the appearance of hypophosphataemia. This deficiency is apparently caused by reduced uptake or increased excretion of phosphate.

The hypo-albuminaemia present during the acute phase of the disease is probably due to albumin seepage into the intestinal lumen via the lesions produced by the worms in the intestinal epithelium. The continued low level in the chronic phase is due to maintained seepage and decreased synthesis because of a decrease in protein absorption.

Although only one infested Merino sheep was included amongst the chronic infestations, it is significant that it reacted less severely than the infested Dorpers. Lapage (1962) states that in South Africa trichostrongyliasis is especially a disease of Persian* sheep, Merinos being less susceptible. The severe reactions of the Dorpers in these experiments and their higher worm burdens when compared with the Merino are probably due to the Somali blood in their breeding.

(3) Pathological anatomy

The lesions in chronic trichostrongyliasis are associated with the secondary effects of the infestation and are not primarily parasitic.

The anorexia and particularly the reduced feed assimilation result in emaciation and atrophy of various organs and fatty changes in the liver and kidneys. The apparent hyposeleniosis causes degeneration of the musculature and myocard. The hypoproteinaemia is reflected in accumulations of fluid in the pericardium, thorax and abdomen, the overall picture being that of sheep suffering from prolonged starvation.

* In South Africa Persian or Black Head Persian Sheep are misnomers. These are in fact Somali sheep.

Summary

Trials are described in which the reactions of 11 sheep infested with *T. colubriformis* and 11 uninfested controls were studied in detail.

The main findings in the acute disease were anorexia, retention of ingesta in the rumen and abomasum, diarrhoea, a severe hypo-albuminaemia and death 16 to 17 days after infestation.

In the chronic disease there was a progressive decrease in feed intake and loss of body weight. Plasma albumin concentration decreased with a rise in plasma gamma globulin concentration later in the disease. A drop in packed red cell volume, haemoglobin concentration, red cell count, total volume of circulating erythrocytes and plasma inorganic phosphate was noted.

There was a decrease in protein, phosphate and possibly selenium uptake leading to emaciation, muscular and myocardial atrophy and degeneration and eventually death.

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