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THE PATHOLOGICAL PHYSIOLOGY OF *OSTERTAGIA CIRCUMCINCTA* INFESTATION

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INTRODUCTION

The pathological physiology of pure *Ostertagia ostertagi* infestations in cattle has been described by several workers. Threkeld & Johnson (1948) recorded a decrease in red cell counts and haemoglobin concentration in experimentally infested calves, while Herlich (1959) found insignificant changes in the blood picture and plasma protein concentrations. Ross, Todd & Dow (1963) found an increase in the pH and decrease in the relative mean pepsin concentration in the abomasa of infested cattle when compared with uninfested controls. Mulligan, Dalton & Anderson (1963) using labelled albumin concluded that hypoalbuminaemia was a lesion of ostertagiasis in cattle.

The present study was undertaken to determine the effect of a pure infestation of *Ostertagia circumcincta* on sheep and a goat kept under laboratory conditions.

METHODS

Merino sheep, bred and reared worm-free and a kid treated with thiabendazole at 100 mg/Kg and thereafter kept worm-free were used in these experiments.

Infective larvae were cultured from faeces collected from sheep infested with a pure strain of *O. circumcincta*. These larvae were diluted with tapwater, counted and divided into the doses required for the various animals. Each individual dose was concentrated on filter paper in a Buchner funnel, this filter paper was placed in a gelatin capsule and dosed to the animal.

Blood analyses were carried out by standard methods as described by Clark (1962) and Horak & Clark (1963). Nitrogen determination was done by standard methods.

Regular faecal egg counts were carried out using a modification of the McMaster technique of Gordon & Whitlock (1939). Where these counts were negative, approximately 5 gm of faeces were mixed with 50 ml of 40 per cent sugar solution and centrifuged; the eggs were removed from the surface of the fluid and examined microscopically.

O. circumcincta were recovered from the intestinal tract and counted as described by Reinecke (1963). In addition the whole intestinal tract was examined to detect any cross-infestation that might have occurred during the course of the experiments.

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Experimental Animals

Trial 1..... Massive infestation followed by challenge. Three Merino sheep.
Sheep 1: Infested with 306,000 larvae and challenged 42 days later with 312,000 larvae; killed 33 days later.
Sheep 2: Infested with 306,000 larvae and challenged 38 days later with 312,000 larvae; killed 33 days later.
Sheep 3: Infested and challenged as for Sheep 2; died 24 days later.

Trial 2..... Single massive infestation. Two Merino Sheep.
Sheep 4: Infested with 270,000 larvae; survived.
Sheep 5: Infested with 270,000 larvae; killed *in extremis* 28 days later.

Trial 3..... Two successive infestations. One Merino sheep and one goat.
Sheep 6: Infested with 170,000 larvae followed by 70,000 larvae five days later; survived.
Goat 1: Infested as for Sheep 6; killed 47 days after second larval dose.

Results

Clinical Observations

(1) *Food intake and nitrogen absorption*

Trials 1 *and* 2

The diet consisted of lucerne hay *ad lib.* The sheep were kept in metabolism cages and the full nitrogen balance was determined.

In Trial 1 the percentage of nitrogen apparently absorbed (i.e. intake minus faecal loss) decreased from an average of 70 per cent in the first week to 45 per cent in the third week after infestation. In Trial 2, however, the nitrogen lost in the faeces exceeded intake during the third week. (The reaction of Sheep 1 was almost identical to that of Sheep 2 so only the latter is shown in Fig. 1).

As the nitrogen content of a prestocked supply of lucerne hay varied little during the experiments, the nitrogen intake as shown in Fig. 1 reflects the food intake as shown in Fig. 2 very closely.

All animals showed a marked drop in food intake during the second and third weeks after initial infestation. In those that survived (Sheep 1, 2 and 4) this was followed by a steady increase in food consumption while those that died (Sheep 3 and 5) showed a temporary increase followed by a decrease (Fig. 1 and 2).

The urine nitrogen loss and total nitrogen balance was determined in Trial 1 from initial infestation to challenge. The average nitrogen balance of the three sheep is shown in Fig. 3.

During the second and third week the animals were in negative nitrogen balance.

Trial 3

The diet consisted of 80 gm yellow maize meal, 4 gm bone meal and 2 gm of salt per day, plus teff hay *ad lib.*

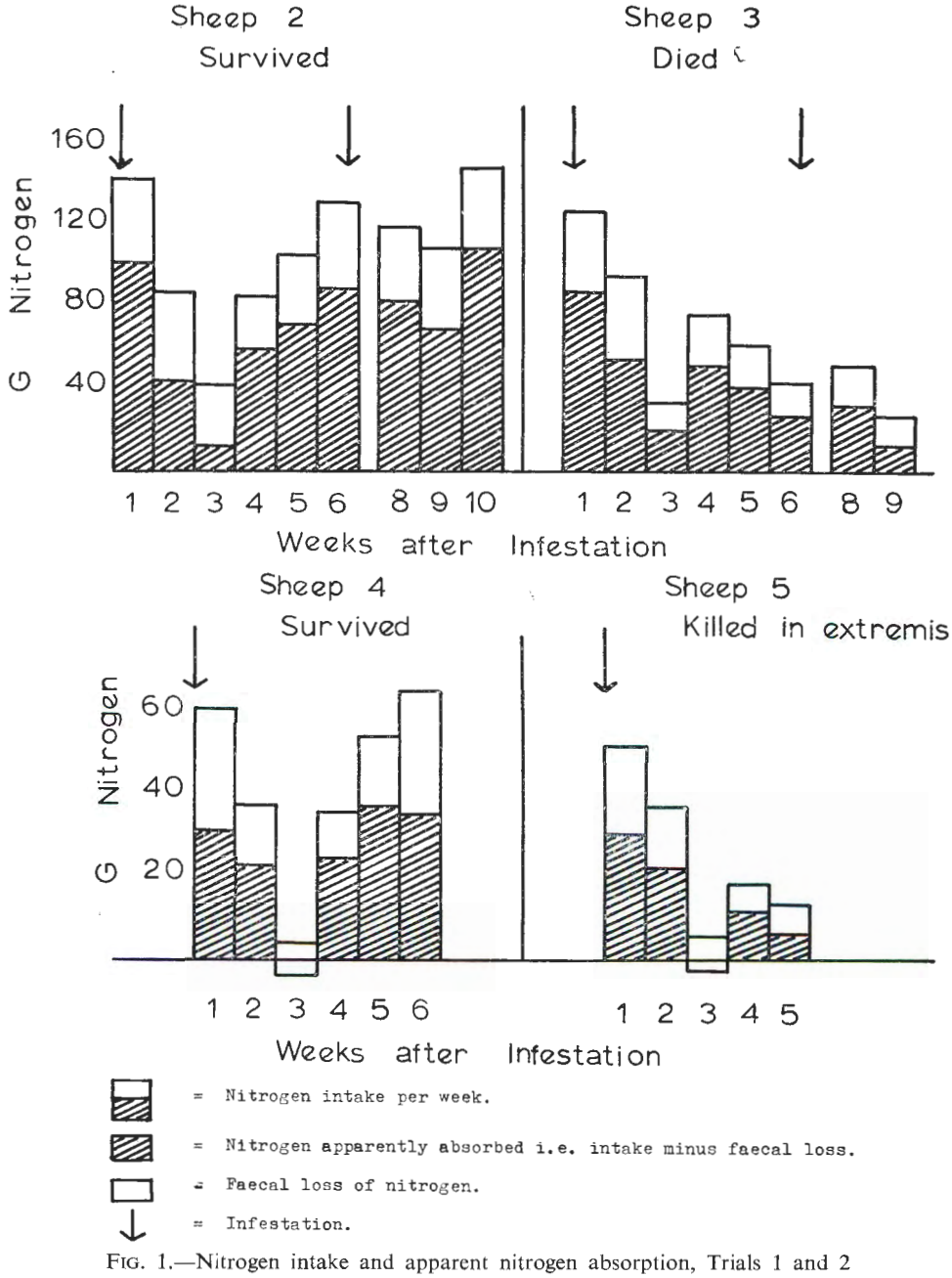

FIG. 1.—Nitrogen intake and apparent nitrogen absorption, Trials 1 and 2

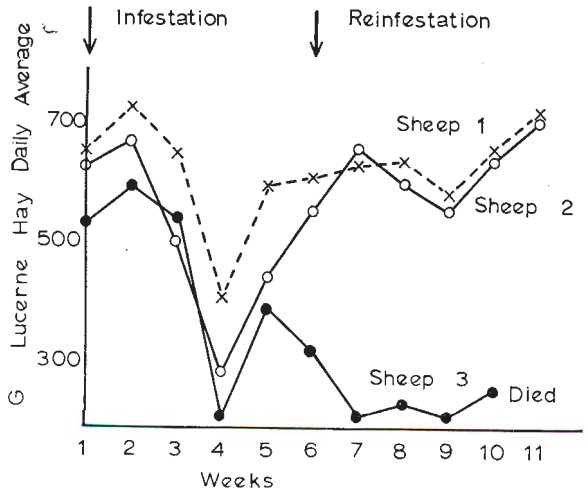

FIG. 2.—Lucerne hay intake, Trial 1

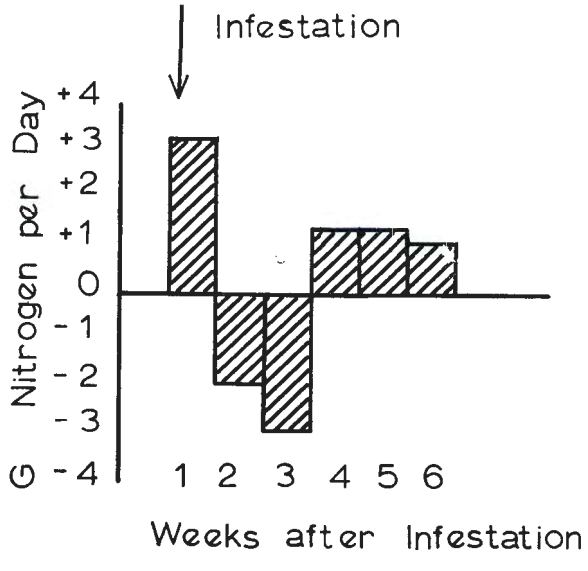

FIG. 3.—Daily nitrogen balance, Trial 1

The animals ate all the maize meal, bone meal and salt throughout the trial; the teff hay intake was little affected by the infestation (Fig. 4).

(2) *Body weight*

In accordance with the findings on the food intake, only Sheep 3 and 5 in Trials 1 and 2 respectively showed any significant weight loss which started within 4 days of infestation as shown in Fig. 5 and 6.

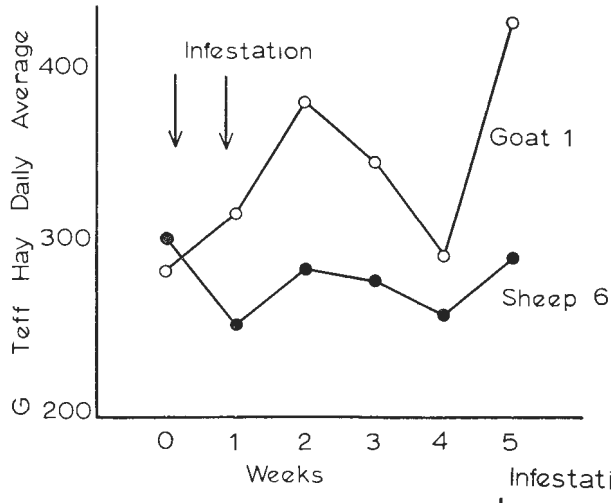

FIG. 4.—Teff hay intake, Trial 3

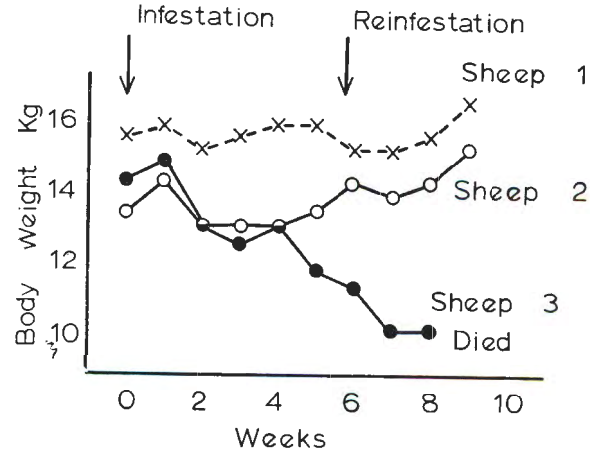

FIG. 5.—Body weights, Trial 1

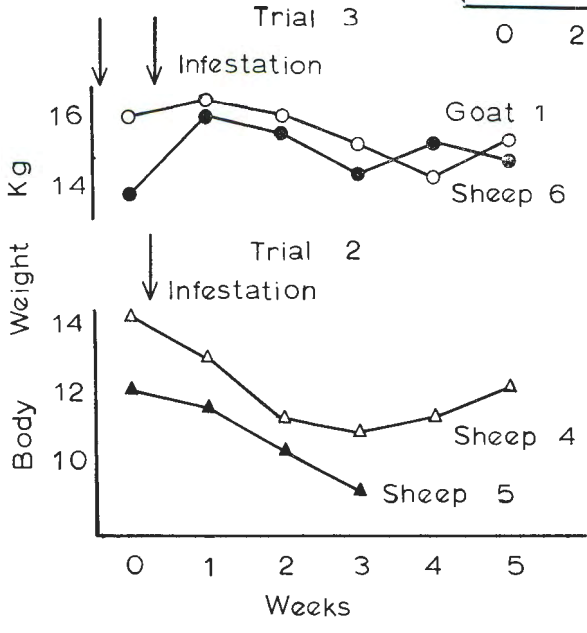

FIG. 6.—Body weights, Trials 2 and 3

(3) *Diarrhoea*

Ten days after initial infestation Sheep 1, 2 and 3 and on the twelfth day Sheep 4 all showed slight diarrhoea with incomplete formation of faecal pellets. Sheep 5 had a severe, fluid, green diarrhoea 24 days after infestation. In all cases the diarrhoea was transient and disappeared within a few days.

(4) *Oedema and anaemia*

Submandibular oedema was observed in Sheep 2 and 3, 24 days, and Sheep 4, 33 days after initial infestation. The oedema disappeared again within three days. Clinical anaemia as evidenced by pale mucous membranes, was seen in Sheep 3 and 5 prior to death and in Sheep 4 at the time of the submandibular oedema and for a week afterwards.

Clinical chemistry

The complete clinical chemistry findings for each of the animals in Trials 1, 2 and 3 are presented in Tables 1, 2 and 3.

(1) *Red blood cells*

All three sheep in Trial 1 showed an initial rise in packed cell volume and haemoglobin concentration for the first two weeks of infestation. This was partly due to plasma volume shrinkage but the absolute volume of circulating red cells rose from an average of 0·37 to 0·50 litre. The four animals in Trials 2 and 3 did not exhibit this phenomenon.

Three to four weeks after infestation all the animals showed a decrease in both packed cell volume and haemoglobin concentration. In Sheep 1 to 3 in which plasma volume was determined, this decrease was not due to haemodilution because the average absolute volume of red cells decreased from the initial figure of 0·37 to 0·31 litre. In Sheep 3 and 5 which succumbed and Sheep 4 which recovered the anaemia was severe. Three out of seven animals therefore showed advanced anaemia. In the others there was little sign of recovery in this respect over the period that the surviving animals were observed.

(2) *Plasma protein concentrations*

The total plasma protein concentration fell in all the experimental animals. The average figure prior to infestation was 6·4 gm per cent while the average of the lowest figures after infestation was 4·9 gm per cent. This fall took place during the first two weeks, i.e. one to two weeks before the red cells showed a decline.

The individual plasma protein fractions were determined in Sheep 1 to 5. The results are summarized in Table 4.

The drop in total plasma proteins was due to a fall in all fractions but the albumins were the most affected. There was no rise in gamma globulin. In the sheep that survived the plasma protein concentrations showed a gradual rise from the third or fourth week onwards (Tables 1 and 2).

TABLE 1.—*Clinical chemistry findings of sheep in Trial 1*

Days after 1st Infestation	—6	1	10	15	22	29	36	48	55	62
Days after 2nd Infestation	—	—	—	—	—	—	—	12	19	26
Packed Cell Volume %	23 26 27	25 28 25	29 31 29	32 34 35	27 29 26	23 22 23	22 23 19	25 28 18	24 24 16	23 25 —
Haemoglobin gm %...	9·5 9·2 9·2	9·7 10·3 9·2	11·3 12·2 11·3	11·3 12·2 11·9	9·7 10·5 10·0	9·2 8·9 8·9	9·2 8·4 7·8	10·0 10·5 7·0	9·2 9·2 5·9	9·2 9·5 —
Plasma Volume (litre)..	1·25 1·02 1·09	1·25 0·99 1·00	1·20 1·00 0·91	1·10 0·97 0·92	1·17 0·97 0·88	1·22 1·18 1·07	1·45 1·03 0·86	1·06 1·03 0·83	1·18 1·03 0·81	1·07 0·94 —
Circulating Red Blood Cell Volume (litre)	0·37 0·35 0·40	0·42 0·39 0·33	0·49 0·45 0·37	0·52 0·50 0·49	0·43 0·40 0·30	0·36 0·33 0·32	0·41 0·31 0·20	0·35 0·40 0·18	0·37 0·32 0·16	0·32 0·31 —
Plasma Albumin gm %	— 2·60 2·52	3·09 — 2·38	— — 2·28	2·03 1·38 1·64	— — —	2·32 1·45 1·53	2·00 1·86 1·60	2·44 2·06 —	2·31 1·83 1·32	2·66 2·17 —
Plasma alpha and beta Globulin gm %	— 1·62 1·15	0·92 — 1·40	— — 1·41	1·41 1·21 1·04	— — —	1·76 1·57 1·39	1·46 1·33 1·08	1·26 1·45 —	1·33 1·09 0·93	1·58 1·34 —
Plasma gamma Globulin gm %	— 2·78 2·33	2·85 — 2·38	— — 1·95	2·20 2·06 1·52	— — —	1·92 2·18 1·64	2·00 2·11 1·72	1·94 1·79 —	2·08 2·18 1·79	1·92 2·39 —
Total Plasma Protein gm %	6·68 7·00 6·00	6·86 6·50 6·16	6·00 5·80 5·64	5·64 4·65 4·20	5·46 4·92 4·56	6·00 5·10 4·56	5·46 5·30 4·40	5·64 5·30 4·56	5·72 5·10 4·04	6·16 5·90 —
Albumin/Globulin Ratio	— 0·59 0·72	0·82 — 0·63	— — 0·68	0·56 0·43 0·64	— — —	0·63 0·40 0·52	0·58 0·54 0·57	0·76 0·64 —	0·68 0·56 0·49	0·76 0·58 —
Blood Sugar mg%.....	53 62 53	57 48 49	51 41 41	41 34 32	53 33 33	44 47 34	61 56 44	52 45 41	47 52 40	58 53 —
Plasma Inorganic PO_4 mg %	— — —	— — —	4·7 5·2 5·9	3·6 3·7 5·4	1·9 1·7 4·5	1·4 4·1 3·7	2·9 6·3 3·7	1·6 3·0 2·3	2·3 2·4 1·7	4·9 4·7 —
Plasma Ca mg %.....	— — —	10·7 8·2 11·4	11·1 10·8 10·8	10·8 10·0 9·2	12·0 11·2 11·2	11·1 10·0 10·0	10·4 10·4 9·3	12·1 11·4 10·7	12·6 11·8 11·1	12·5 10·0 —
Plasma Mg mg %.....	1·71 2·00 2·28	2·41 2·28 1·85	1·28 2·00 2·14	1·85 1·57 1·75	2·00 1·71 2·00	0·57 1·00 2·14	0·57 1·14 2·00	2·14 1·85 2·85	— — —	— — —

The figures for Sheep 1, 2 and 3 are given respectively under each heading

TABLE 2.—*Clinical chemistry findings of sheep in Trial 2*

Days after Infestation.....	0	6	13	20	27	29	34	41
Packed Cell Volume %.....	29 30	22 24	28 25	28 25	23 16	— 14	18 —	19 —
Haemoglobin gm %.....	10·0 10·5	9·2 8·9	10·3 9·2	10·0 8·9	9·2 6·5	— —	6·7 —	7·3 —
Plasma Albumin gm %.....	2·70 3·00	— —	— —	2·50 2·20	— 2·17	— —	2·07 —	— —
Plasma alpha and beta Globulin gm %	1·60 1·70	— —	— —	1·20 1·30	— 0·77	— —	1·01 —	— —
Plasma gamma Globulin gm %.....	1·90 1·10	— —	— —	1·60 1·40	— 2·70	— —	2·22 —	— —
Total Plasma Protein gm %.....	6·16 5·80	6·00 5·80	5·64 5·80	5·30 4·92	5·64 5·64	— 5·10	5·30 —	6·60 —
Albumin/Globulin Ratio.....	0·77 1·10	— —	— —	0·89 0·81	— 0·63	— —	0·66 —	— —
Blood Sugar mg %.....	63 59	59 47	44 41	29 29	36 30	— 67	51 —	49 —
Plasma Inorganic PO_4 mg %.....	5·2 4·4	4·8 5·4	4·2 3·4	4·5 5·0	1·0 1·6	— 2·8	1·1 —	2·3 —

The figures for Sheep 4 and 5 are given respectively under each heading

TABLE 3.—*Clinical chemistry of the sheep and goat in Trial 3*

Days after 1st Infestation...	—2	5	12	19	26	33	40
Packed Cell Volume %.....	24 32	25 31	25 29	23 27	24 27	22 25	23 24
Haemoglobin gm %.....	7·8 10·8	9·2 11·3	8·4 10·3	8·7 10·3	8·4 10·0	8·7 10·3	8·1 9·4
Total Plasma Protein gm %	6·32 7·00	6·50 6·82	6·00 6·32	5·30 5·64	5·30 5·64	5·46 5·80	5·64 6·50
Blood Sugar mg %.....	51 51	45 51	41 51	43 43	44 48	46 48	36 42
Plasma Inorganic PO_4 mg %	5·5 5·5	3·3 5·9	4·5 4·4	5·2 4·5	4·8 7·4	5·2 7·1	4·9 7·2

The figures for Sheep 6 and Goat 1 are given respectively opposite each heading

TABLE 4.—*Relative changes in the plasma protein fractions*

Protein fractions	Initial gm %	At lowest T.P.P. gm %	% Drop
Albumins	2·8	1·9	32·1
α & β Globulin	1·4	1·3	7·1
γ Globulin	2·2	1·7	22·7
T.P.P.	6·4	4·9	23·4
A/G Ratio	0·78	0·63	—

T.P.P. = Total plasma proteins
A/G = Albumin/Globulin ratio

(3) *Plasma volume and total circulating plasma proteins*

These reached their lowest levels 15 to 22 days after infestation (Table 5).

TABLE 5.—*Plasma volumes and plasma protein levels of sheep in Trial* 1

Sheep	Initial Figures			At lowest Plasma Volume			
	Pl. Vol.	T.P.P.	T.C.P.P.	Pl. Vol.	T.P.P.	T.C.P.P.	Days
1	1·25	6·68	84	1·10	5·64	62	15
2	1·02	7·00	71	0·97	4·65	45	15
3	1·09	6·00	63	0·88	4·56	40	22

Pl. Vol. = Plasma volume (litre)
T.P.P. = Total plasma proteins (gm %)
Days = Days after infestation on which lowest Pl. Vol. was recorded
T.C.P.P. = Total circulating plasma proteins

(4) *Blood sugar*

All the infested animals showed a drop in blood sugar level during the second and third weeks, but in no case was frank hypoglycaemia exhibited. (In this laboratory the normal range for blood sugar concentration in sheep is taken to be 35 to 60 mg per cent while figures below 20 mg per cent are considered frankly hypoglycaemic). In the animals which survived, the blood sugar levels tended to rise again after the third week. Even in Sheep 3, which died, the final figure was well within the normal range (40 mg per cent). Sheep 5, which also succumbed, showed no signs of recovery except for a terminal sharp rise. Such a terminal rise in blood sugar level has been observed in sheep dying from a variety of diseases.

(5) *Plasma inorganic phosphate*

One of the most remarkable findings was a sharp drop in the plasma inorganic phosphate which occurred in all the animals except those in Trial 3. In this laboratory the normal range is taken as 3 to 9 mg per cent. The findings are summarized in Table 6.

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TABLE 6.—*Plasma inorganic phosphate levels mg per cent*

Sheep No.	Initial	Lowest	Final
1	4·7 (10)	1·4 (29)	4·9 (62)
2	5·2 (10)	1·7 (22)	4·7 (62)
3	5·9 (10)	1·7 (55)	1·7 (55) Died
4	5·2 (0)	1·0 (27)	2·3 (41)
5	4·4 (0)	1·6 (27)	2·8 (29) Died

Figures in brackets indicate days after infestation

Plasma inorganic phosphates were at their lowest levels 22 to 55 days after infestation.

(6) *Plasma calcium*

The total plasma calcium was not significantly affected but showed some tendency to rise as the inorganic phosphate dropped.

(7) *Plasma magnesium*

No deviation from normal was noted.

Faecal examinations

Trial 1: These are summarized in Table 7. A temporary drop occurred in the faecal egg counts one to two weeks after larval challenge.

TABLE 7.—*Average weekly egg counts in Trial* 1

Sheep No.	Day first egg seen	Week challenged	Average egg count during week								
			1–2	3	4	5	6	7	8	9	10
1	23	6	0	0	87	76	147	400	10	76	134
2	19	6	0	19	162	67	200	167	19	86	134
3	24	6	0	0	57	86	173	67	49	80	Died

Trial 2: The first egg was seen in the faeces of Sheep 4, 34 days after infestation; 42 days later it had 300 eggs per gram (e.p.g.); this was a pure *Ostertagia* infestation based on larval differentiation. At the time of its death, 28 days after infestation, Sheep 5 had as yet passed no eggs in its faeces.

Trial 3: Sheep 6 and Goat 1 both had faecal egg counts in the fifth week after initial infestation. When the trial ceased 76 days after initial infestation Sheep 6 had a pure *Ostertagia* count of 400 e.p.g.

Worms recovered at autopsy

Sheep 3 and 5 had died during the course of the experiments. Sheep 1 and Goat 1 were slaughtered 75 and 52 days after initial infestation respectively. Sheep 2 was treated with Thibenzole at 50 mg/Kg 65 days after initial infestation; a faecal bag was attached daily and the sheep was slaughtered six days after treatment. The total number of worms recovered from the sheep and the goat and from the faecal bags of Sheep 2 are shown in Table 8.

TABLE 8.—*Worms recovered at autopsy*

Sheep No.	*O. circumcincta*			Cross-infestation			
	Stage of development			*Trichostrongylus* spp.	*Oesophagostomum columbianum*	*Trichuris* spp.	*Skrjabinema* spp.
	4th	Adult	Total				
1	18	1,423	1,441	1,170	1	6	0
2	160	13	173	483	0	0	0
3	478	29,108	29,586	69	1	1	0
5	2,010	5,760	7,770	0	0	0	0
Goat 1	4,827	9,248	14,075	0	0	0	245

Relatively small numbers of *O. circumcincta* were recovered from Sheep 1 and 2 while a fair number of *Trichostrongylus colubriformis* were present. Large numbers of *O. circumcincta* were recovered from Sheep 3 and 5 and Goat 1 and very mild cross-infestations with other nematode species were present.

Autopsy findings

The following macroscopic changes were noted in varying degrees in all the animals that died or were slaughtered: Slight serous atrophy of the mesenteric fat, especially along the abomasum and duodenum, numerous nodules in the abomasum, particularly in the pyloric region, shallow erosions containing helminths in the fundic region. In the sheep that died and the one slaughtered *in extremis*, anaemia and splenic atrophy were also noted.

DISCUSSION

Clinical observations

Anorexia is one of the first symptoms in many helminth infestations. It has been noted in fascioliasis (Sinclair, 1962), oesophagostomosis (Bremner, 1961) and paramphistomiasis (Horak & Clark, 1963), and in the present trials it was observed soon after infestation. During the early phase of the disease *O. circumcincta* damages the abomasal mucosa mechanically by migration and growth within the lumen of the gastric glands (Sommerville, 1954). This results in functional disturbances such as decreased pepsin concentration and increased pH as noted by Ross *et al.* (1963). These factors may cause the anorexia.

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The decrease in percentage apparent nitrogen absorption may be due to: (i) a decrease in absorption of nitrogenous constituents from the food ingested; (ii) a decrease in reabsorption of such substances normally passing into the digestive tract from the body; and (iii) an increased passage of nitrogenous substances such as plasma proteins and mucus into the intestinal tract. The work of Mulligan *et al.* (1963) indicates that there may well be increased loss of plasma albumin through the intestinal mucosa but the fact that the actual faecal nitrogen loss fell markedly during the period of anorexia does not indicate a marked loss of " endogenous " protein in the faeces.

That the anorexia and effect on body weight was due to the effects of the infestation and not the conditions under which the sheep were kept is shown by the fact that a control sheep, kept under identical conditions gained 2·7 Kg over the experimental period.

Anaemia

No explanation can be offered for the initial rise in circulating red blood cells shown by the three sheep in Trial 1. As red cell counts were not made, the exact nature of the anaemia shown in the second and third weeks post-infestation cannot be determined but the close correlation between packed cell volume and haemoglobin concentration would appear to exclude a macrocytic anaemia. Ross (1963), however, found no significant change in the packed red cell volume of calves suffering from ostertagiasis, while Threkeld & Johnson (1948) found a drop in haemoglobin concentration.

Plasma proteins

As the periods of lowest nitrogen absorption and lowest total plasma protein concentration more or less correspond, the hypoproteinaemia cannot be ascribed to reduced synthesis as a result of protein deficiency. It is of interest to note that the alpha, beta and gamma globulins were reduced, contrary to the findings of Ross (1963) who recorded a rise in the globulin concentration in infested calves.

Oedema

From the evidence to hand, the oedema noted can only be ascribed to the hypoproteinaemia, yet in a previous experiment on paramphistomiasis in sheep oedema did not develop at plasma protein levels below 4 gm per cent (Horak & Clark, 1963), a figure lower than any recorded in the present trials. Factors other than hypoproteinaemia may well play a role in the pathogenesis of oedema in helminthiasis.

Blood sugar

The drop in blood sugar level can probably be ascribed directly to the low food intake.

Plasma inorganic phosphate

No explanation can be offered for the remarkable drop in plasma inorganic phosphate recorded in the infested animals.

General

The anaemia and oedema present made it impossible to distinguish the condition clinically from severe haemonchosis. This fact emphasizes the importance of faecal culture and egg or larval differentiation in the diagnosis of helminth infestations.

Helminthology

The prepatent period of *O. circumcincta* is considered to be 19 to 21 days. Excepting for Sheep 2 this period was prolonged, presumably due to the severity of the infestation. After reinfestation there was a temporary depression of egg count in the sheep in Trial 1. From the low egg counts and high worm-burdens obtained from some of the animals it must be concluded that *O. circumcincta* is not a very prolific egg layer. Similar observations were made by Michel (1963) in ostertagiasis of calves.

The challenge dose of larvae resulted in " self-cure " in Sheep 1 and 2, if this had not already taken place at the time of challenge. Sheep 3, however, which received the same infesting and challenge doses, succumbed and harboured a large number of worms. The total number of worms recovered from Sheep 2 may be less than the actual total at the time of treatment, as *O. circumcincta* are digested during their passage through the bowel (Reinecke, 1963).

The course of the infestation in Trial 3 was influenced by the second infesting dose as neither of the animals reacted severely to infestation. The goat's food consumption decreased after initial infestation but increased two days after reinfestation indicating some interference with the course of the disease. The large number of fourth stage worms recovered from the goat also indicates some degree of resistance in this animal.

The cross-infestation with *Trichostrongylus* spp. does not seem to have affected the clinical chemistry results as identical findings were obtained from those animals in which no cross-infestation was present.

From the large number of *O. circumcincta* larvae necessary to produce death it can be concluded that this worm is not highly pathogenic.

Autopsy

Sommerville (1954) described the pathology of ostertagiasis in sheep; the macroscopic appearance of the abomasum was similar to that noted by Sommerville. The plasma proteins are lost presumably by seepage via the shallow erosions in the abomasum.

General

Decrease in food consumption, nitrogen absorption, blood sugar and plasma protein concentration is associated with the disease as caused by the immature worms where the effects are mainly mechanical. A decrease in packed red cell volume, haemoglobin concentration, volume of circulating red blood cells, and plasma inorganic phosphate level occurs when the worms mature and the effects are parasitic.

Summary

1. Trials are described in which six sheep and one goat were artificially infested with *O. circumcincta* and their reactions studied in detail.

2. The main pathological findings were:—

(i) Marked decrease in packed red cell volume, haemoglobin concentration and circulating red blood cell volume.

(ii) A sharp drop in plasma inorganic phosphate which occurred in five of the seven animals.

(iii) Anorexia, decreased apparent nitrogen absorption and weight loss or retarded weight gain.

(iv) A decrease in plasma albumin, gamma globulin and total circulating plasma proteins.

3. Egg counts were low even with large worm burdens.

4. On reinfestation two sheep cleared themselves of the original and challenging infestation.

5. Large numbers of larvae were necessary to cause death.

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