

## STUDIES ON PARAMPHISTOMIASIS. V. THE PATHOLOGICAL PHYSIOLOGY OF THE ACUTE DISEASE IN SHEEP

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### INTRODUCTION

From a study of the literature it would appear that very little work has been published on the pathological physiology of this infestation. Subsequent to the completion of this work Lengy (1962) reported on the biochemical and haematological changes in one sheep infested with paramphistomes.

### METHODS

Merino sheep bred and reared under worm-free conditions were used throughout. This ensured that the experimental animals were not suffering from other intercurrent infestations.

Infestation was carried out as follows: Metacercariae of the conical fluke *Paramphistomum microbothrium* Fiscoeder, 1901, were collected and counted on cellulose strips as described by Swart & Reinecke (1962), and stored till required (Horak, 1962 a). The strips were dosed in gelatine capsules, the infestation comprising  $170,000 \pm 5,000$  metacercariae.

Blood analyses were carried out by standard methods as detailed by Clark (1962). An "Eel" apparatus and scanner and an "Ott" planimeter were used for the electrophoretic fractionisation of the plasma proteins.

Paramphistomes were recovered from the intestinal tract as described by Horak (1962 b). The methods of mixing and sampling of the ingesta for counting purposes were similar to those described by Reinecke, Snijders & Horak (1962) for anthelmintic trials on nematodes. Counting was carried out macroscopically by adding water to the sample to a volume of approximately 1 litre, mixing well and pouring into a black photographic developing tray ( $24 \times 18 \times 3$  in) with coloured parallel lines drawn 2 in apart on its base. The pale pink worms were readily seen and counted against the black background.

Four different experiments were carried out.

#### *Experiment 1: Preliminary trial*

##### *Method*

Three sheep (No. 1, 2 and 3) were infested and blood samples were analysed weekly.

##### *Results*

All three sheep died from 22 to 27 days later. As shown in Table 1 gross infestation was found at autopsy, proving that the animals had suffered from acute paramphistomiasis.

STUDIES ON PARAMPHISTOMIASIS. V

In the blood analyses the following factors showed no significant changes even shortly before death: Red cell osmotic fragility; red cell sedimentation rate; concentrations of blood creatinine, urea, plasma sodium, potassium, magnesium, inorganic phosphate and chloride.

The total plasma protein concentration showed a progressive drop from the 14th day after infestation till death, the average initial and final figures being 5.9 and 3.3 gm per cent respectively. The blood calcium concentration fell over the same period.

The plasma volume (Evan's Blue method) showed a terminal drop from 70 ml/Kg body weight to 66 ml/Kg. The red cell counts, haematocrit values and haemoglobin concentrations showed a terminal rise corresponding to the shrinkage in plasma volume. When the absolute volume of circulating erythrocytes was calculated it was found to have risen by 40 per cent in one case, dropped by 7 per cent in another and remained constant in the third.

Eosinophiles disappeared from the peripheral blood in the final stages.

Figures showing significant changes are shown in Table 2.

The body weights of the sheep are shown in Table 3.

TABLE 1.—*The number of paramphistomes recovered from sheep in four experiments*

Sheep No.	Number of M/C* dosed	Paramphistomes recovered	Percentage of M/C recovered as paramphistomes	Percentage of flukes anterior to pylorus	Percentage of flukes in first three metres of intestine	Percentage of flukes distal to first three metres
1	169,000	72,145	42.7	8.8	87.6	3.6
2	175,000	70,139	40.1	7.9	91.1	1.0
3	167,000	87,768	52.6	8.4	89.3	2.3
4	170,000	40,039	23.6	13.6	85.1	1.3
5	170,000	46,897	27.6	9.3	89.2	1.5
†6	170,000	66,556	39.2	5.7	19.2	75.1

*Number of paramphistomes recovered from sheep given two doses of metacercariae*

(1) Number recovered after the initial dose

7	169,000	2	—	100.0	} These sheep were treated with "Lintex".	—
8	174,000	370	—	100.0		—
9	25,000	5,752	23.0	100.0		—
10	50,000	9,445	18.9	100.0		—
11	75,000	7,388	9.9	49.8		50.1

(2) Number recovered after the challenge dose

7	200,000	53,476	26.7	3.5	45.5	51.0
8	204,000	18,162	8.9	4.7	94.1	1.2
9	200,000	13,673	6.8	20.0	77.6	2.4
10	202,000	18,341	9.1	26.7	70.9	2.4
11	202,000	75,273	37.3	7.3	92.4	0.3

\*M/C = Metacercariae

†This autopsy was conducted some while after death

TABLE 2.—Average figures from sheep in Experiment 1

	Days after infestation				
	0	7	14	21	24
Haematocrit (%).....	27.0	27.0	28.0	30.0	31.0
Plasma vol. (ml/Kg).....	72.0	69.0	—	70.0	66.0
Total Plasma Proteins (gm %)	5.9	6.1	4.5	3.7	3.3
Plasma Ca (mg %)	10.9	9.7	9.7	8.7	7.0

TABLE 3.—Body weights (Kg) (Experiment 1)

Sheep	Days after infestation				
	0	7	14	21	24
1.....	12.2	12.6	11.3	11.3	—
2.....	14.0	14.9	13.1	12.8	11.3
3.....	25.7	24.8	24.1	24.5	23.2

The findings at autopsy were: Generalised emaciation, ascites, hydrothorax and oedema of the lungs, massive infestation of the first three metres of the small intestine with immature paramphistomes with localised serous atrophy of mesenteric fat along the affected portion of the small intestine, hyperaemia and oedema of the infested intestinal wall, petechiae and shallow erosions on the intestinal mucosa with numerous immature conical flukes deeply embedded in the mucosa. Sheep No. 3 did not show pulmonary oedema despite a final total plasma protein concentration of 3.3 gm per cent. This animal showed haemorrhage into the adrenal cortex.

#### *Experiment 2: Food and water consumption and electrophoresis of plasma proteins*

##### *Method*

This was conducted in the same way as Experiment 1 except that food and water consumptions were recorded and the plasma proteins were fractionated by electrophoresis.

Three sheep (No. 4, 5 and 6) were infested and the factors listed in Experiment 1 were again determined and the previous results confirmed.

##### *Results*

*Food consumption.*—Lucerne hay only was offered *ad lib.* All three sheep showed a progressive anorexia commencing on the 6th to 7th day post infestation (see Fig. 1).

STUDIES ON PARAMPHISTOMIASIS. V

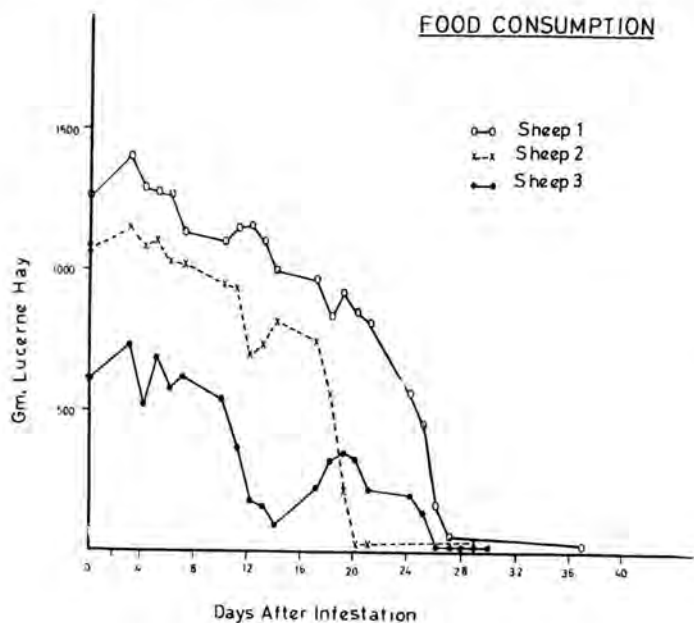


FIG. 1.—Experiment 2.  
Food consumption

*Water consumption.*—The water consumption remained more or less constant. As sheep on a low food intake usually consume correspondingly less water (Clark & Quin, 1949), the intake during the period of anorexia can be considered abnormally high.

*Diarrhoea.*—All three sheep showed a foetid diarrhoea with sudden onset from 26 to 28 days after infestation and persisting till death.

*Death.*—All three cases ended fatally on the 30th, 28th and 36th day post infestation respectively.

*Autopsy.*—The findings were as in Experiment 1. No haemorrhage into the adrenal cortex was seen. A submandibular oedema, however, was present in sheep No. 5.

*Chemical Pathology.*—The findings in regard to the plasma proteins are presented in Table 4 and the average figures in Fig. 2.

The marked drop in total plasma protein concentration occurring between the 14th and 21st day was confirmed. Analysis of the electrophoretograms revealed that this loss was almost exclusively confined to the albumin fraction.

The plasma volume of sheep No. 4 rose slightly despite a marked drop in plasma albumin. Sheep No. 5 and 6 showed a decrease in plasma volume.

TABLE 4.—*Plasma proteins and blood and plasma volumes (Experiment 2)*

Sheep	Days after Infestation	Alb.* gm %	$\alpha$ and $\beta$ Glob.† gm %	$\gamma$ Glob. gm %	Total plasma Prot. gm %	Plasma Vol. (litre)	Blood Vol. (litre)	Plasma Ca mg %
4.....	7	—	—	—	—	1.16	1.66	9.6
	10	2.97	0.62	1.15	4.74	—	—	—
	14	2.53	0.84	1.19	4.56	1.13	1.64	10.5
	21	0.95	1.47	0.92	3.34	—	—	9.7
	24	—	—	—	—	1.25	1.74	—
	28	0.41	0.93	1.48	2.82	1.25	1.78	6.7
Died.....	30	—	—	—	—	—	—	—
5.....	0	2.63	1.56	1.97	6.16	1.81	2.66	11.0
	7	—	—	—	6.00	1.67	2.61	10.4
	10	2.54	1.16	1.60	5.30	—	—	—
	14	3.13	0.94	1.57	5.63	1.65	2.62	11.6
	21	0.90	1.87	0.93	3.70	—	—	9.2
	24	—	—	—	—	1.72	2.71	—
Died.....	28	0.82	1.63	1.25	3.70	1.42	2.41	8.6
6.....	0	3.20	1.36	2.12	6.68	1.90	2.79	12.1
	7	—	—	—	—	2.05	3.06	9.2
	10	2.77	1.55	1.32	5.64	1.94	2.85	—
	14	3.26	1.39	1.35	6.00	—	—	12.1
	21	1.37	1.73	1.10	4.20	—	—	10.6
	24	—	—	—	—	2.20	3.10	—
	28	0.83	1.91	1.12	3.86	1.84	2.79	8.3
	32	1.04	1.58	1.24	3.86	1.59	2.65	6.8
	34	—	—	—	3.86	1.60	2.54	8.7
	Died.....	36	—	—	—	—	—	—

\* Alb. = Albumin

† Glob. = Globulin

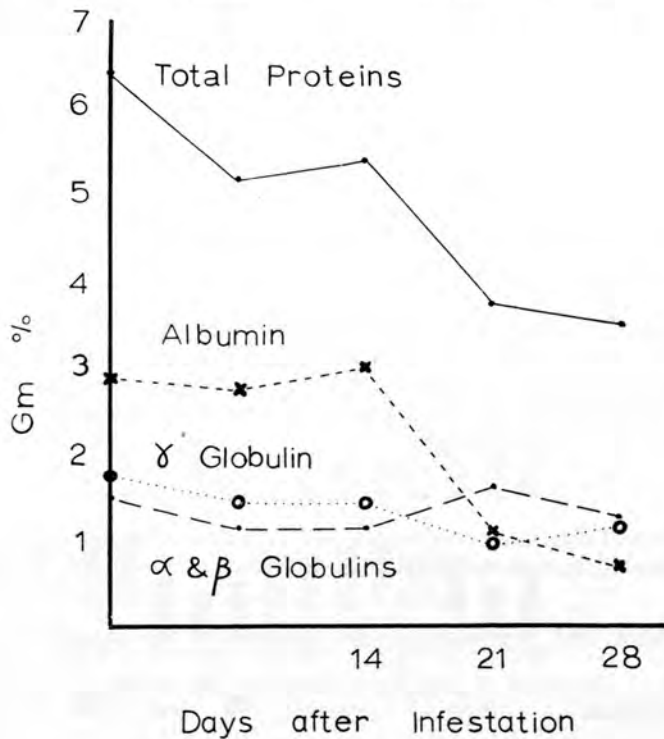


FIG. 2.—Experiment 2. Average plasma protein concentration

STUDIES ON PARAMPHISTOMIASIS. V

The plasma calcium again decreased with the proteins.

The findings with regard to the blood cells will be found in Table 5. Sheep No. 5 and 6, i.e. those which showed a decrease in plasma volume, showed a rise in haematocrit, red cell count and haemoglobin concentration. That this was not entirely relative to the plasma volume shrinkage is indicated by a rise in calculated absolute volume of red cells.

One sheep (No. 6) showed a terminal leucocytosis.

TABLE 5.—*The blood cells (Experiment 2)*

Sheep No.	Days after infestation	Haematocrit %	Red cell count (million)	Hb. gm %	Total Red cell volume (ml)	Leucocyte count (1,000)
4.....	0	28	10.4	10.8	550	6.5
	7	30	11.1	13.2	500	9.0
	14	31	12.9	11.6	510	9.0
	21	28	10.0	11.6	490	—
	28	30	—	12.4	530	—
5.....	0	32	12.7	12.2	850	7.0
	7	36	12.8	13.5	940	8.0
	14	37	14.7	13.5	970	6.0
	21	39	15.0	14.6	990	8.0
	28	41	—	16.7	990	—
6.....	0	32	11.4	12.2	890	9.3
	7	33	13.0	13.2	910	10.5
	14	32	12.2	12.7	910	8.8
	21	28	11.8	13.5	900	8.0
	28	34	—	—	950	—
	36	37	15.0	14.0	940	16.8

*Experiment 3: Recovery after anthelmintic treatment*

*Method*

In this experiment two sheep (No. 7 and 8) were infested as before but were treated after the onset of symptoms with N-(2'chlor-4-nitrophenyl)-5-chlorsalicylamid\* at the dosage rate of 50 mg/Kg body weight (Horak 1962 b). The sheep were treated 18 and 25 days after infestation when their total plasma protein concentrations had fallen to 3.08 and 4.04 gm per cent respectively.

*Results*

Sheep No. 7 and 8 showed diarrhoea on the 16th and 19th days after infestation respectively. This disappeared spontaneously on the 20th day in sheep No. 8 to recur on the 22nd day.

The effects of infestation and treatment on the appetites of the animals are shown in Fig. 3.

\* "Lintex", Agro-Chem (Pty.) Ltd.

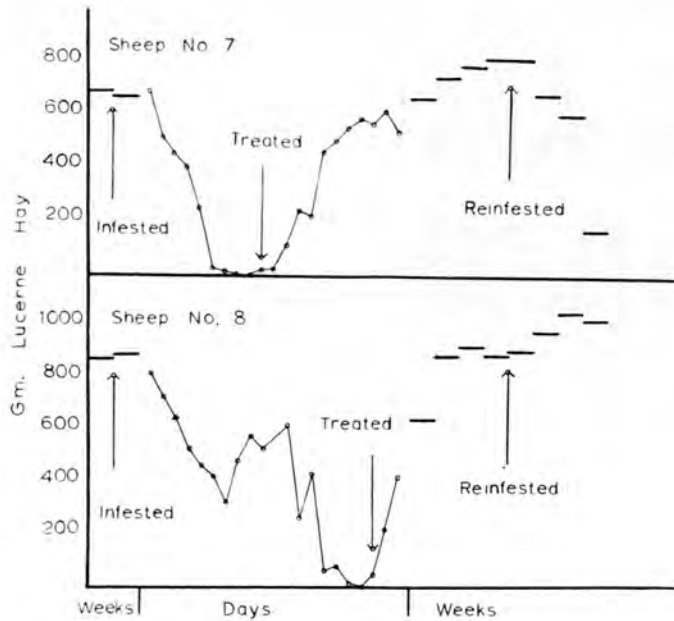


FIG. 3.—Experiment 3. Food consumption. (Solid lines represent average intake for one week. Lines connected through circles represent daily intake.)

In both cases the appetite decreased suddenly from the 8th day after infestation and recovered rapidly after treatment, reaching normal levels within one week. The diarrhoea disappeared in both sheep within three days of treatment.

The findings in regard to blood and plasma volumes and plasma proteins are shown in Fig. 4 and 5, and the plasma protein electrophoretograms of sheep No. 8 during various stages of the disease are shown in Fig. 6.

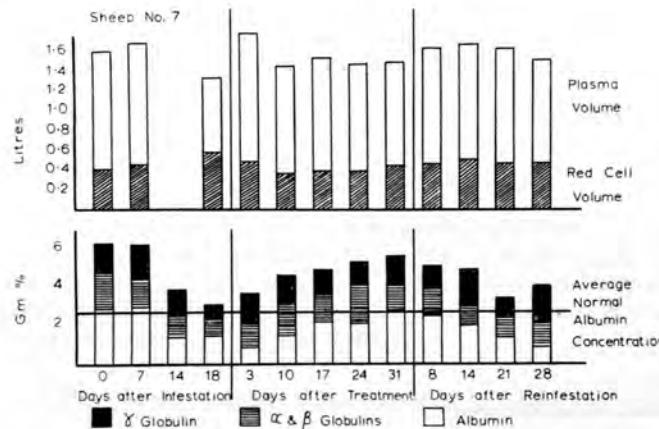


FIG. 4.—Experiment 3. Blood and plasma volumes and plasma protein concentration

STUDIES ON PARAMPHISTOMIASIS. V

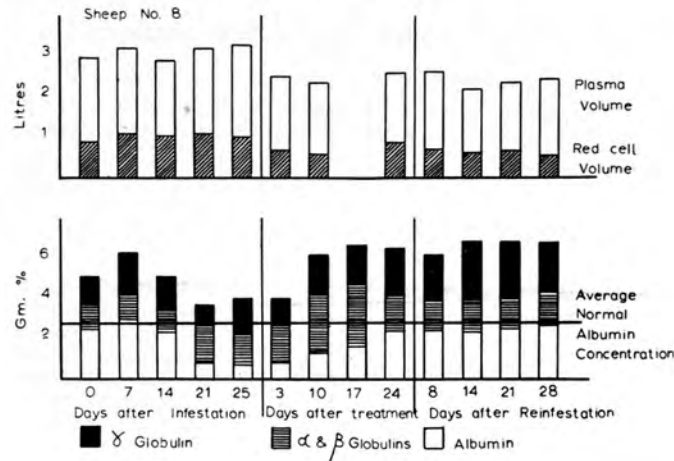


FIG. 5.—Experiment 3. Blood and plasma volumes and plasma protein concentration

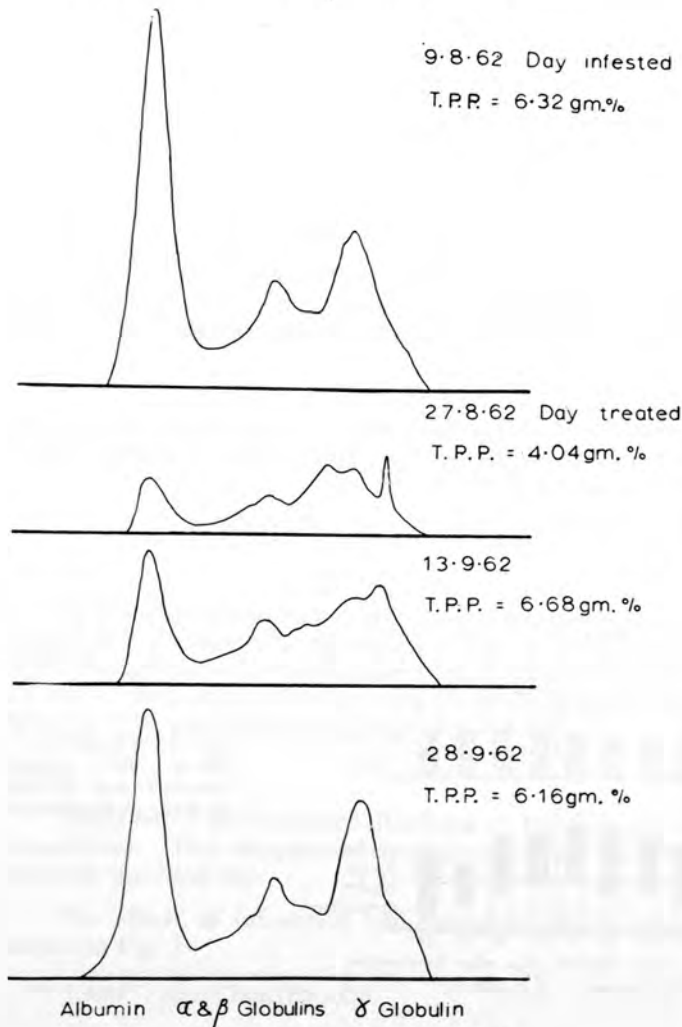


FIG. 6.—Experiment 3 Electrophoretograms of sheep No. 8. (Read from the left.)



As in the previous experiments both sheep showed a marked drop in plasma albumin concentrations from the seventh day post infestation. After treatment there was a steady recovery.

Forty-nine days after the first infestation and 31 and 24 days after treatment sheep No. 7 and 8 were challenged with 200,000 and 204,000 metacercariae respectively.

During the second week after reinfestation sheep No. 7 showed a progressive decrease in food intake. It developed diarrhoea on the 25th day and was slaughtered three days later. As will be seen from Fig. 4, the total plasma protein concentration was somewhat low (5.64 gm per cent) when the second infestation took place. This figure dropped progressively to 3.34 gm per cent with a slight rise, not wholly accounted for by plasma shrinkage, in the last week. The autopsy findings were: Slight ascites and hydrothorax; serous atrophy of the mesenteric fat along the affected portion of the intestine; hyperaemia, oedema, petechiae and shallow erosions were present in the first six metres of the small intestine and were not confined to the first three metres as in the previous autopsies; massive invasion by immature flukes of the first six metres of the small intestine. Two paramphistomes only from the initial infestation were recovered from the rumen.

Sheep No. 8 showed little or no reaction to the reinfestation either as regards food intake or plasma protein concentration. It was slaughtered for autopsy on the 32nd day after reinfestation and the findings were as follows: The autopsy was negative except for milder intestinal lesions than described for the previous cases. The number of paramphistomes recovered was also relatively small when compared with the other sheep. In the rumen and omasum 370 immature fluke from the initial infestation were found (see Table 1).

#### *Experiment 4: Effects of reinfestation*

##### *Method*

Three sheep were infested with the following numbers of metacercariae, viz:—

- Sheep No. 9—25,000
- Sheep No. 10—50,000
- Sheep No. 11—75,000

After an interval of 63 days the animals were challenged with 200,000 to 202,000 metacercariae each.

##### *Results*

The reactions to the initial infestations were as follows: No diarrhoea was observed in any of the sheep and no loss of body weight was recorded in sheep No. 9 and 10. Sheep No. 11 originally weighed 38 Kg and showed a progressive weight loss from the 23rd day after infestation. At the time of challenge, 63 days after the initial infestation it had lost 6.6 Kg.

The plasma protein concentrations of these three sheep are shown in Fig. 7.

STUDIES ON PARAMPHISTOMIASIS. V

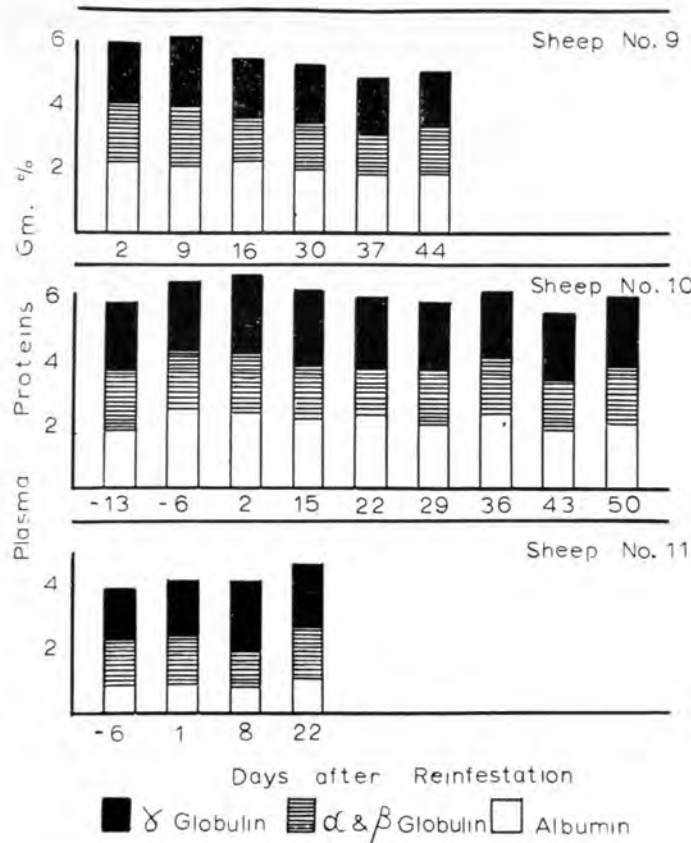


FIG. 7.—Experiment 4. Plasma protein concentration

Sheep No. 9 and 10, which had received the lighter initial infestations and which had shown total plasma protein concentrations of over 5 gm per cent at the time of reinfestation, showed no reactions. They were slaughtered 48 and 50 days after reinfestation respectively and the autopsy findings were practically identical to those of sheep No. 8. A larger number of flukes from the initial infestation were recovered however (see Table 1).

The third sheep (No. 11), which had initially received 75,000 metacercariae, had a total plasma protein concentration of only 3.86 gm per cent when reinfested. Despite the fact that this figure rose slightly over the next 22 days, the animal died at the end of that period. Intermittent diarrhoea was seen from the 14th day after challenge. The autopsy findings were similar to those seen in the highly susceptible sheep No. 1 to 6. Paramphistomes from the initial infestation had not as yet completed their forward migration and a large percentage of them were still present in the small intestine (see Table 1).

*Controls*

1. *Plasma proteins*

*Method.*—Twelve plasma samples from apparently normal sheep were analysed to determine plasma protein content, both to collect "normal" figures and to control the experimental findings.

*Results.*—Albumin  $2.72 \pm 0.28$ , alpha and beta globulins  $1.57 \pm 0.20$ , gamma globulins  $2.10 \pm 0.44$  and total plasma proteins  $6.39 \pm 0.45$  gm per 100 ml plasma. The albumin/globulin (A/G) ratio was  $0.76 \pm 0.11$ .

## 2. Effect of "Lintex" on erythropoiesis

*Method.*—After treatment with "Lintex" both sheep, No. 7 and 8 showed a drop in circulating erythrocytes (Fig. 4 and 5). In order to determine whether the drug had any effect on erythropoiesis, eight normal sheep were subjected to the same level of dosage.

*Results.*—Four days later the average haematocrit value had fallen from 28.4 to 25.5 per cent. Almost all the animals showed a slight drop but one sheep showed a marked fall from 31 to 20 per cent. Eleven days after dosing the average haematocrit was 26.6 per cent but the one sheep was still low at 22 per cent. The reaction of this one sheep may well have been fortuitous.

## 3. Anthelmintic

*Method.*—To confirm the efficacy of the drug "Lintex", faecal collecting bags were attached daily to sheep No. 7 and 8 for three days after treatment.

*Results.*—The total numbers of worms recovered from the faeces of these sheep were 8,550 and 23,233 respectively.

## DISCUSSION

### *Pathological Physiology*

As the most significant changes were found in the plasma proteins, this aspect will be discussed first.

Dukes (1955) cites the following figures for sheep (gm per cent): Total 5.74, albumin 3.07, serum globulins plus fibrinogen 2.67, giving an albumin/globulin ratio of 1.39. Deutsch & Goodloe (1945) report an A/G ratio of 0.8 and a figure of  $0.76 \pm 0.11$  was recorded for the control sheep in this study.

Deutsch & Goodloe (1945) also comment on the poor resolution of components in sheep plasma by electrophoresis. As will be seen from Fig. 6 fair resolution was obtained in the present experiments but the separation of alpha and beta globulins and fibrinogen was not achieved.

Lengy (1962) reported that the plasma proteins varied within normal limits in one lamb infested with 75,000 metacercariae. The results now reported show that the most significant lesion in acute paramphistomiasis is a marked drop in plasma protein concentration involving the albumin almost exclusively. This might be caused by direct loss or by reduced synthesis. Margen & Tarver (1956), using isotopic techniques, have established the half life of plasma albumin in humans to be 26 days. From this they calculate that, assuming the rate of catabolism remains constant, a 50 per cent drop in the rate of synthesis will result in a 20 per cent drop in the plasma albumin concentration in 16 days. In the present experiments the treated infested sheep took from 21 to 28 days to restore some 50 per cent of their plasma albumin, indicating a similar rate of turnover as in the human. The infested sheep, however, lost over 50 per cent of their circulating albumin in seven days, which cannot be ascribed to defective synthesis alone. There must therefore be either a direct loss,

presumably through the parasitised gut wall, or increased catabolism. During Experiment 2 an attempt was made to carry out nitrogen balance trials but the copious watery diarrhoea made separation of faeces and urine impossible. At the present stage it can therefore only be postulated that the albumin is lost through the parasitised intestinal wall.

An interesting point is the extremely low plasma protein concentrations found. Hawk, Oser & Summerson (1947) state that, in humans, oedema almost invariably occurs when the total plasma proteins fall below the critical level of 5·3 gm per 100 ml. Sheep in the present experiment failed to show marked oedema with a total plasma protein concentration of below 4 gm and an albumin concentration of less than 1 gm per 100 ml.

It was also found that the plasma volume did not tend to shrink significantly until the total plasma concentration fell to below 4 gm per cent. After this there was a reduction in plasma volume which tended to mask any further fall in protein concentration.

The results show conclusively that there is no tendency to anaemia. Not only a relative but also an absolute increase in red cell volume was encountered. Lengy (1962) recorded a slight drop in haematocrit, haemoglobin concentration and red cell count.

The absence of any detectable disturbance in plasma electrolyte balance or concentration despite the copious watery diarrhoea is remarkable.

There is no evidence of any disturbance in kidney or liver function.

The actual cause of death has not been established but it would appear to be likely to be due to a terminal sudden collapse of the heart due to blood volume shrinkage and oedema of the lungs.

As pointed out above, infested animals showed a tendency towards an absolute increase in circulating erythrocytes. This may be explained as a response to anoxia as a result of retarded circulation due to plasma shrinkage. It will be seen from Fig. 4 and 5 that the animals treated with "Lintex" both showed a subsequent drop in circulating erythrocytes and consequently the eight normal sheep were treated with "Lintex" at the same dosage level.

Although this test did indicate some reduction in haematocrit values after dosing with "Lintex", this would not appear to be of clinical significance and certainly does not outweigh the remarkable efficacy of the drug.

#### *Pathology*

The lesions caused by the immature paramphistomes during their sojourn in the small intestine are of prime importance in the pathology of this condition. The fluke attaches itself to the intestinal wall by means of a plug of mucosa held fast within the terminal sucker or acetabulum. The muscular sphincter of the acetabulum causes strangulation and pressure necrosis of the enclosed tissue and when the flukes release their hold to migrate forward to the rumen these necrotic areas form erosions, from which presumably protein seepage takes place.

Some of the immature flukes become deeply imbedded in the mucosa and are often visible just below the intestinal serosa. Their return to the intestinal lumen must also cause considerable damage to the mucosa. These findings are similar to those described by Boray (1959).

### *Helminthology*

No sexually mature paramphistomes resulting from the infestation with metacercariae were found in any of the sheep. The total number of paramphistomes recovered from sheep No. 1 to 6 varied between 40,039 and 87,768. Of these immature flukes 5.7 to 13.6 per cent were found anterior to the pylorus in all cases, in sheep No. 1 to 5, 85.1 to 91.1 per cent were present in the first three metres of the small intestine while the remaining 1.0 to 3.6 per cent were present distal to the first three metres (see Table 1). The paramphistomes present in sheep No. 6 were spread throughout the small intestine but not attached anywhere, possibly because this autopsy was carried out quite a while after death. The occurrence in this sheep of a large number of fluke in the caecum and colon, however, would suggest a spontaneous clearance of parasites just before death.

The fact that in all cases a relatively small percentage, but considerable actual number, of young flukes were recovered anterior to the pylorus at death, suggests that the commencement of massive forward migration is the trigger mechanism for the final collapse of the host.

### *Anthelmintic*

The number of worms recovered from sheep No. 7 and 8 after treatment cannot be taken as accurate indices of the worm burdens at that time, as many paramphistomes are digested during their passage through the intestinal tract after treatment (Horak 1962 b).

The two paramphistomes from the initial infestation recovered from the rumen of sheep No. 7 and the 370 recovered from the rumen and omasum of sheep No. 8, were most probably already there at the time of treatment as "Lintex" does not affect paramphistomes in these organs when dosed *per os* in fluid medium (Horak 1962 b).

### *Resistance*

A dose of  $170,000 \pm 5,000$  metacercariae had proved to be lethal in the six sheep allowed to go the full course of the disease. In order to ascertain whether sheep developed any resistance to reinfestation a larger dose ( $200,000 \pm 5,000$  metacercariae) was chosen as a challenge in Experiments 3 and 4.

On reinfestation sheep No. 7 showed slight evidence of resistance as the course of the disease was protracted when compared with the original infestation. A large number of the young flukes were attached in the second three metres of the small intestine whereas the majority normally occur only in the first three metres, a considerable number were also recovered from the caecum and colon suggesting spontaneous evacuation of the infection. The percentage of metacercariae (26.7 per cent) which developed into immature flukes, however, is consistent with the percentages recovered from highly susceptible sheep (Table 1, sheep No. 1 to 6). No resistance to the course of the disease except protraction was evident as the plasma proteins fell and the sheep had to be slaughtered as death appeared imminent.

Sheep No. 8, 9 and 10 were not only partially resistant to reinfestation but showed only slight temporary decreases in plasma proteins. The percentages of the reinfesting doses of metacercariae recovered as paramphistomes were 8.9, 6.8 and 9.1 per cent respectively which are well below the figures for the highly susceptible sheep No. 1 to 6. Flukes from the original infestations were recovered from the fore-stomachs of these three sheep (see Table 1).



## STUDIES ON PARAMPHISTOMIASIS. V

Sheep No. 11, which had not recovered from the effects of the initial infestation, as evidenced by the low plasma protein level and loss of weight, reacted as an entirely susceptible sheep would on challenge, 42.7 per cent of the challenge dose being recovered as young flukes. Flukes from the initial dose had as yet not completed their forward migration as many were recovered from the small intestine (see Table 1)

It would appear that resistance to reinfestation is stimulated by the presence of flukes in the rumen (sheep No. 8, 9 and 10). If, however, the sheep has not recovered from the effects of the initial infestation this resistance does not develop (sheep No. 11).

### *General*

Observations made on several sheep suffering from acute paramphistomiasis indicated polydipsia, sheep frequently stood for long periods with their mouths immersed in the water. If infected sheep on pasture behave similarly the increased thirst would keep them close to the water source and increase the chance of infection as the grazing around the water and the water itself would be the source of infection.

An interesting observation which has been made by farmers is that the blood of sheep slaughtered because of paramphistomiasis appeared very thick. The reason for this phenomenon has now been shown to be due to the terminal drop in plasma volume and an actual increase in some cases of the volume of circulating red cells.

### SUMMARY

Experiments are described in which eleven sheep were artificially infested and their reactions studied in detail.

The main lesion was found to be a marked decrease in plasma albumin concentration with a resultant shrinkage in plasma volume.

There was no indication of anaemia. Electrolyte balance and liver and kidney function were unimpaired.

Anorexia was the first symptom exhibited, commencing approximately a week after infestation. A characteristic foetid diarrhoea appeared some two weeks later.

The efficacy of N-(2'chlor-4-nitrophenyl)-5-chlorosalicylamid in the treatment of this infestation was strikingly demonstrated.

The main pathological lesion was pressure necrosis and erosion of the intestinal mucosa caused by the acetabular sphincter of the fluke.

In all cases death occurred only when a considerable number of fluke had commenced their forward migration to the rumen.

In highly susceptible sheep the percentage of metacercariae recovered at autopsy as immature paramphistomes was greater than the percentage recovered from resistant sheep after reinfestation.

Resistance appeared to be stimulated by the presence of fluke in the rumen.

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