THE PATHOGENESIS OF FLYSTRIKE IN SHEEP

INTRODUCTION

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Approximately 3 million sheep in Australia die from flystrike annually. These losses represent a large economic drain on the industry and it is therefore surprising to find that very little work has been carried out to delineate the events preceding the death of sheep from flystrike. This paper describes our studies of the physiological indices of sheep suffering from flystrike, and the haematological, bacteriological and pathological changes which occur during challenge and which may contribute to the death of some affected animals. It also reports the effects of graded levels of challenge on fibre production by sheep.

We have pooled our efforts from within various disciplines and have presented an overview of what actually happens to sheep suffering myiasis. We believe that this preliminary work will provide a further dimension to our understanding of flystrike, and would welcome collaborative involvement by workers in other disciplines who share a common interest in this subject.

PHYSIOLOGICAL ASPECTS OF CHALLENGE BY LUCILIA CUPRINA

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MATERIALS AND METHODS

In order to examine the physiological events associated with challenge we studied larval development in sheep carrying afferent vascular catheters serving the area of skin where larvae were implanted. To do this we devised a technique for catheterising a cutaneous vessel serving an area of skin on the flank (Hoey and Hopkins 1983) and enclosed this area in a canvas sleeve securely affixed to the skin. This enabled the site to be implanted with specific numbers of first instar larvae during simultaneous manipulation of plasma profiles in the afferent vasculature.

Administration of anti-histamine (tripelennamine hydrochloride 60 mg/day), histamine (50 mg/day), betamethasone soluble phosphate ester (3 mg/day) and a prostaglandin inhibitor (acetylsalicylic acid 500 mg/day) through the vasculature permitted larval survival and growth to be monitored in the presence of these treatments. Animals were catheterised bilaterally to observe larval development in test and control sites on the one sheep. Test sites were ipsilateral to treatment infusions; the contralateral (control) side was infused with saline. Infusion rates were maintained at c. 150 ml/day. These studies were supported by in vitro experiments in which the test substances were added to larval culture systems. Flies were field strain Lucilia cuprina bred in laboratory colonies for less than three generations.

Autoradiographic measurements of wool growth were made to determine the effect of larval challenge on this production parameter. Four Merino wethers were given whole body doses of 100 µCi L-(^{35}S)-cysteine to measure wool production during periods associated with challenge. The challenge consisted of implanting 500 first instar L. cuprina larvae each day for 8 days.

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RESULTS

Table 1 indicates the marked extent to which tripelennamine hydrochloride, histamine, betamethasone and acetylsalicylic acid suppressed larval development. It also shows that histamine administration enhanced larval development. These data support the contention that the humoral response to challenge may involve the release of substances which can in turn influence subsequent events. The resultant 48% fly survival from control implants of 200 larvae is in keeping with levels of larval survival in non-catheterised control sheep (O'Donnell et al. 1980). The in vitro studies examining larval development in cultures containing up to 0.5 mg/ml of test substances clearly showed that these compounds had no direct toxic effects on the larvae.

TABLE 1 The effects of local infusions of tripelennamine hydrochloride, histamine, betamethasone and acetylsalicylic acid on larval development

Treatment	Larvae		Pupae	Flies
	implanted	surviving	surviving	survival rate (%)
Control (8)	200	102	97	48
Tripelennamine hydrochloride (2)	200	30	27	13
Histamine (2)	200	134	130	65
Betamethasone (2)	200	19	17	8
Acetylsalicylic acid (2)	200	49	46	23

Figures in brackets indicate number of observations

All four sheep in the wool growth study suffered a depression of fibre production during treatment and post-treatment periods. The percentage change from the initial control period indicates that the greatest depression occurred during the 30 days after larval challenge (Table 2).

TABLE 2 Wool production (μm^3/fibre/day) of four individual sheep before, during and after larval challenge

No. of fibres examined	Mean fibre production (μm^3/fibre/day) ± SE					
	Period I (24 days)		Period II (10 days)		Period III (30 days)	
	Mean	SE	Mean	SE	Mean	SE
59	143	5	122	4 (-15)	108	3 (-24)
54	162	5	140	5 (-13)	122	4 (-25)
54	130	6	122	4 (- 6)	105	4 (-19)
59	102	3	69	2 (-32)	63	1 (-38)

Figures in brackets indicate the percentage change from control (Period I) values

DISCUSSION

The evidence that acetylsalicylic acid, anti-histamine and betamethasone infusions suppressed larval development, and the detection of an opposite effect from histamine, prompt some discussion of the extent to which prostaglandin, histamine and/or endogenous glucocorticosteroids may be involved in the normal

expression of physiological changes associated with challenge. Both prostaglandin and histamine are capable of mediating vascular changes which increase capillary fragility and enhance leakage of plasma proteins which are in turn a vital component of substrate for larval development. The general role of both substances and glucocorticosteroids in the progression of inflammatory responses suggests that they could be at least partly responsible for the generation of a micro-environment which is conducive to larval development. Sheep with an innate resistance to challenge may be somewhat anergic to challenge and may not summon the responses which provide this micro-environment. The practical impact of this hypothesis awaits elucidation.

The changes observed in fibre morphology were mainly the result of a diminution of fibre diameter; longitudinal growth did not change markedly. During microprojection a tapering of fibres was observed at the end of the treatment period. The wool from one animal was subsequently found to be frankly tender and could be removed by gentle traction. Samples of wool from this sheep showed a high percentage of brush-ended fibres, indicating that the fleece was in the process of being shed. This phenomenon was probably the result of increased circulating cortisol levels elicited in response to the stress associated with blowfly strike since glucocorticosteroids are the only compounds known to induce this change in fibre morphology (McDonald et al. 1982; Panaretto 1979). In a previous communication we showed that synthetic glucocorticosteroid when administered at 0.5 mg/kg/day decreased the area of myiasis resulting from a standard challenge (O'Donnell et al. 1980). This effect was thought to be an expression of the anti-inflammatory properties of the steroid. Other work done in this laboratory has shown that doses as high as 2.8 mg/kg/day of the same glucocorticosteroid are necessary for brush-end formation (McDonald et al. 1982). Hence, the level of endogenous glucocorticosteroid secretion by an animal responding to challenge could at least partly characterise that individual as physiologically resistant or susceptible to challenge.

Larval challenge resulted in a marked depression in wool production in all four sheep not only during challenge but also during the 30 day post-treatment period. The sustained drop in production represented a decrease in total wool production which, on current values, would mean an economic loss of approximately 50 cents per sheep. Previous assessments of the losses due to blowfly strike have not included this consideration and may have to some extent underestimated the cost of this parasite to the industry.

The marked reduction in fibre diameter during and after challenge must surely reflect a reduction in the tensile strength of the staples of sheep suffering from flystrike. This finding could unveil some unexpected ramifications in relation to the selling of wool by descriptive terms including tensile strength. Any discounts received for wool objectively demonstrating a reduction in tensile strength may prompt a re-evaluation of the importance of blowfly control under field conditions.

FEVER AFTER LARVAL CHALLENGE

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MATERIALS AND METHODS

This paper describes a study of the clinical indices in groups of sheep challenged with graded doses of Lucilia cuprina larvae. Eighteen Merino wethers were maintained in metabolism cages and fed 600 g lucerne pellets daily throughout the study. Sheep were allowed a 4 week settling period before any measurements were taken. The experiment commenced with a 7 day pre-treatment period in which

normal physiological indices were recorded. A treatment period followed during which 500 first instar L. cuprina larvae were applied daily to each of four wethers in three groups until each animal had been implanted with a total of 4000 larvae (group A), 3000 larvae (group B) or 1500 larvae (group C).

Two sheep were maintained as untreated controls for each group. The first day on which larvae were implanted was designated as day 0. The graded challenges initiated an average area of myiasis of 400 to 800 cm^2 which is in keeping with the extent of myiasis commonly experienced under field conditions. Groups A and C were implanted during summer and group B during winter.

Feed consumption, live weight, respiration rate and pulse rate were recorded daily for each animal. Rectal temperature was measured three times daily at 8 a.m., 1 p.m. and 5 p.m.

RESULTS

Cardinal signs, feed intake and liveweight change are presented graphically in Fig. 1. as group mean values. All groups of treated sheep displayed a marked elevation of rectal temperature. In group A the rise was more rapid, higher, and sustained for a longer period than in the other groups. At least one animal in each of the groups reached a peak temperature in excess of 41°C. Rectal temperatures invariably fell as the number of larvae remaining on the animals decreased.

The respiration rates of all sheep in groups A and C initially increased then subsided as larval numbers diminished. There was no overall increase in the respiration rate of sheep in group B. Pulse rates were elevated in all groups though the extent of the change was markedly greater in the animals carrying the greatest larval challenge. The control sheep exhibited no significant change in rectal temperature, respiration or pulse rate.

Complete anorexia occurred in individual animals at all levels of challenge but the effect on appetite was most marked in the sheep in group A which were infested with the greatest number of larvae. Subsequent liveweight loss was also greatest (mean 6 kg) in group A.

DISCUSSION

The rapid increases in rectal temperature and respiration rate initiated by larval implantation are evidence of a systemic reaction to challenge. The fact that most sheep reached peak temperatures well in excess of 40°C indicates that they were markedly febrile. The level of fever may have been increased had the sheep been depastured in conditions where severe climatic heat stress was present and/or where they had to graze extensively to satisfy feed and water requirements. Rectal temperatures and respiration rates both declined as the numbers of larvae on sheep decreased. The metabolic changes were probably associated with the absorption of toxin by the sheep: either exogenous toxin produced by larvae and/or bacteria or an endogenous toxin resulting from tissue damage or a combination of both.

Anorexia with subsequent liveweight loss occurred early in the larval challenge and continued progressively during infestation. It was not until the larvae had left the animals that increases in feed intake and liveweight change were observed. This also suggests that the transient presence of toxins in the sheep's circulation may be the major factor predisposing to the debilitating effects of strike.

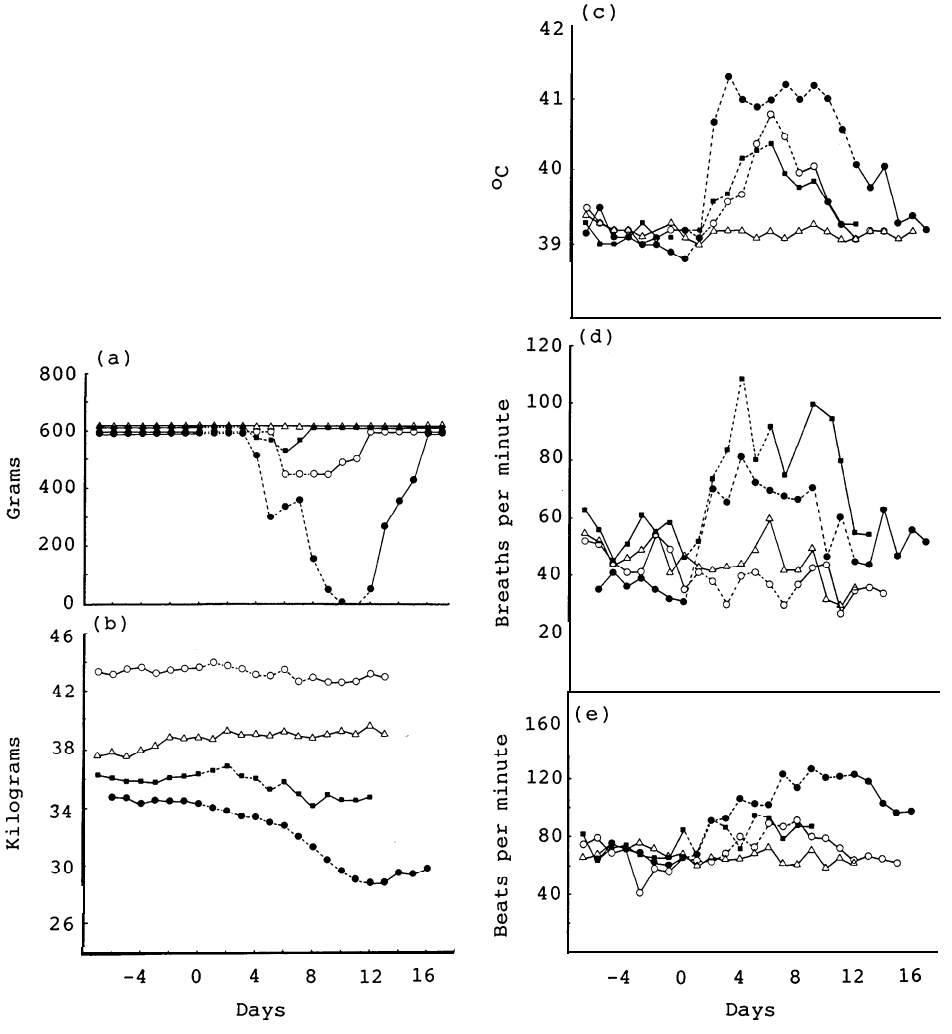

Fig. 1. Group mean values for (a) feed intake; (b) liveweight; (c) rectal temperature; (d) respiration rate; (e) pulse rate for sheep subjected to implantation with Lucilia larvae: group A, 4000 over 8 days ● ; group B, 3000 over 6 days ○ ; group C, 1500 over 3 days ■ ; controls ▲ . The broken line denotes the period of larval challenge.

HAEMATOLOGICAL CHANGES IN SHEEP SUFFERING FROM BLOWFLY STRIKE

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MATERIALS AND METHODS

Haematological studies were carried out on the eight sheep from groups A and B which were challenged with 4000 and 3000 Lucilia cuprina larvae respectively and on two control animals (see earlier paper "Fever after Larval Challenge").

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Samples were collected from all sheep pre-experimentally and on alternate days during the implantation period and until recovery from clinical effects or death.

Values for haemoglobin (Hb), packed cell volume (PCV) and differential white cell counts were determined using standard techniques (Dacie and Lewis 1968) and for total red (RCC) and white (WCC) cell counts using a Coulter Electronic cell counter. Tests for defects in the coagulation system included prothrombin and activated partial thromboplastin times, fibrinogen estimation and the detection of fibrin/fibrinogen degradation products (FDP).

RESULTS

Two sheep from group A were severely depressed and were euthanased for autopsy on day 14; the other two animals in this group had returned to normal when euthanased on day 18. The implanted sheep suffered a depression in Hb values to levels which reflect a marginally anaemic state (mean 11.1 g/dl to 8.0 g/dl). Control animals registered minimum values of 9.7 g/dl which is still within the normal range. Values for PCV and RCC paralleled these and there was no morphological evidence of a regenerative response to the fall in Hb values.

Abnormalities were detected in the coagulation system of one infested sheep and it was in group A. On day 15, prothrombin (14.6 sec) and activated partial thromboplastin (31.5 sec) times in that animal were less than control values (16.5 sec and 35 sec respectively): the fibrinogen level had fallen from a high value of 5.5 g/l (normal value 2.0-3.0 g/l) on day 9 to 1.0 g/l; the test for FDP was positive and very few platelets were present in the blood smear. Bone marrow smears showed an increase in the number of megakaryocytes and in the myeloid:erythroid ratio. The values for the other nine sheep remained within normal limits.

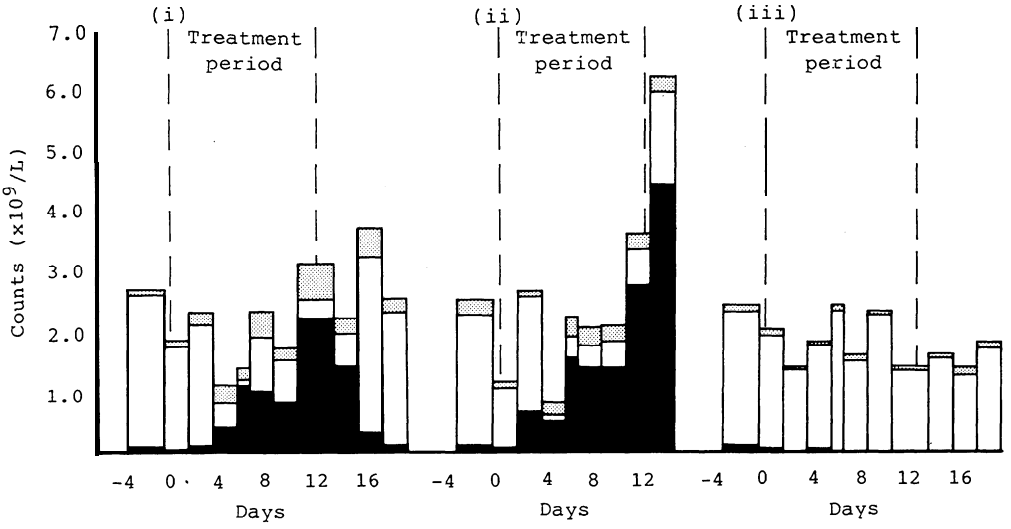

Fig. 1. Mean leucoyte values for two moderately affected sheep (i); two severely affected (ii); two controls (iii). Treated sheep were implanted with 4000 Lucilia larvae over 8 days. Segmented neutrophils ❑ ; band neutrophils ■ ; monocytes ◘.

The changes in leucocyte values for the sheep in group A are presented in Fig. 1. In all four treated animals there was an increase in circulating neutrophils on day 2. By day 4 there was a marked reduction in these cells (neutropenia) and the few cells present showed degenerative toxic changes. In the two severely affected sheep the total number of neutrophils gradually increased to levels above normal, whence there was a high percentage of juvenile forms (band neutrophils), and toxic changes were still apparent (Fig. 1 ii). In the two remaining infested sheep the response, although slightly less, was similar in pattern to that of the severely affected sheep until day 11. Subsequently values gradually returned to almost normal distribution by the final sampling on day 18 (Fig. 1 i). There was also a monocytosis in all four infested animals during the period of larval challenge. No significant changes occurred in leucocyte value in the controls (Fig. 1 iii). All four sheep in group B survived and had returned to clinical normality by day 12 when sampling was discontinued. The only significant changes were in leucocyte values. All sheep demonstrated an increase in circulating neutrophils on day 3. Neutropenia was present in three of the four sheep on day 7 but transient toxic changes were observed in only two of these sheep. Subsequently there was a progressive increase in total neutrophil values with a decrease in the proportion of juvenile forms until the final sampling on day 12.

DISCUSSION

Clear evidence of a severe. systemic challenge to the implanted sheep was reflected in the haematological responses. Initially there was a marked increase in circulating neutrophils, the cells involved in the host's defence mechanisms. By day 4 the number of these cells circulating had decreased, suggesting mobilisation to the site of tissue damage. Subsequently a marked progressive increase in total neutrophils and monocytes occurred in all infested sheep (Fig. 1 i and ii).

In the two severely affected sheep in group A the increase in neutrophils after day 5 was mainly in juvenile forms and toxic changes were apparent until the animals were autopsied. This sequence of changes is indicative of the presence of a severe bacterial or toxic challenge which elicited a marked neutrophilia with a shift to the left as the animal was attempting to maintain high circulating levels of white cells responsible for combating such a challenge. In the other two sheep in group A a regenerative process was reflected in the neutrophil values (Fig. 1 i) indicating that these animals elicited a myeloid response capable of withstanding the toxic challenge. This laboratory finding was supported by the observation that both of these sheep recovered when larval numbers diminished after day 11.

In one of the four sheep there was evidence prior to death of haemostatic defects indicating the formation of intravascular thrombi. This was confirmed at autopsy when changes consistent with disseminated intravascular coagulation (DIC) were revealed. DIC is a syndrome secondary to many severe disease processes, in which activation of the coagulation cascade results in utilisation of fibrinogen and platelets, deposition of fibrin in the micro-circulation and generation of fibrin degradation products. Bacterial endotoxins of gram negative organisms such as Pseudomonas and the release of thromboplastic substances from damaged tissues are just two of the wide range of factors that may initiate DIC (Hardaway 1966). The haematological diagnosis of DIC in one of the experimental sheep was confirmed by the histological findings of microthrombi in the capillaries and arterioles of the heart, kidney and lung. Toxins capable of initiating DIC are likely to be associated with larval attack so that this syndrome may be a contributory factor in deaths from blowfly strike.

PATHOLOGICAL CONSEQUENCES OF MYIASIS IN SHEEP

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MATERIALS AND METHODS

Part of the sequence of investigations into the pathogenesis of blowfly strike embraced a study of the bacteriological and pathological changes associated with myiasis in sheep.

Bacteriology

Skin and wool samples were collected from two treated animals and one control on days 0, 3, 4, 7, 10, 13 and 16 (Merritt and Watts 1978). These samples were monitored for total bacterial counts. Because of the association of Pseudomonas SPP. with fleece rot, centrimide-containing media selective for that genus were used and specific Pseudomonas counts were made as myiasis developed.

Blood samples were collected from severely affected animals by jugular venipuncture and cultured for 7 days in order to isolate and identify any bacteria present. Tissue from major organs was cultured aerobically at autopsy.

Pathology

Skin biopsies were taken from the area of myiasis on four sheep which were implanted with 500 Lucilia cuprina larvae daily for 8 days. The samples were taken on alternate days between 1 and 13 in order to monitor the inflammatory changes associated with strike. All sheep were autopsied between 14 and 18 days after the commencement of challenge. Two control animals were also autopsied. Tissue sections were processed and stained by routine histological methods for microscopic examination. Mallory's phosphotungstic acid haematoxylin (PTAH) stain was used to demonstrate the presence of fibrin microthrombi.

RESULTS

Bacteriology

Total bacterial counts on the skin rose from 10^3-10^4/cm^2 to 10^8-10^9/cm^2 within 3 days of infestation and remained at that level until day 13 while total counts on the control sheep remained less than 10^4/cm^2.

Total bacterial counts on the wool rose from 10^7/g before infestation to 10^9-10^{11}/g within 3 days of implantation and remained at that level until day 13. These cultures contained a variety of organisms including Bacillus spp., Proteus spp., enterobacteriacae, micrococci and streptococci. Pseudomonas spp. were found to rise from less than 300 to 10^5-10^6/cm^2 on the skin and from less than 1000 to 10^6-10^8/cm^2 on the wool.

Blood samples taken from two animals on day 12, 2 days prior to death, yielded no bacteria. Bacteriological samples of major organs from all sheep were cultured aerobically with negative results.

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Pathology

Skin biopsies taken on day 1 indicate that the larvae had already evoked an acute exudative dermatitis characterised by massive neutrophil infiltration with ulceration and necrosis of the epidermis. The keratin layer of the epidermis (stratum corneum) was denuded and in areas where the ulceration extended through the epidermis there was leakage of tissue fluid, neutrophils and red blood cells onto the skin surface. In areas not ulcerated there was a tenfold thickening of the epidermis due to hyperplasia of the stratum germinativum (acanthosis). The sub-cutis and dermis were hyperaemic and oedematous. By the third day the lesion was more severe and early proliferation of fibroblasts had begun in the sub-cutis.

From day 5 through to day 13 the nature of the inflammatory infiltrate changed from an acute, predominantly neutrophilic response to a chronic, predominantly mononuclear response involving lymphocytes, histiocytes and plasma cells. Regeneration of the stratum corneum had occurred by day 7, and by day 13 the epidermis was hyperkeratotic. Healinq of the lesion was accompanied by pronounced fibrosis of the underlying dermis and sub-cutis. Gram stains of skin sections revealed a marked increase in the number of gram negative and gram positive bacteria associated with the strike lesion compared to control skin sections.

At autopsy the regional prescapular lymph nodes were enlarged and serum drainage tracts were present down the inside of the forelegs. One sheep exhibited other gross pathological changes. Several pale areas 2 to 10 mm in diameter were seen on the surface of both kidneys. In sections the areas were wedge shaped and confined to the renal cortex. Irregular pale areas were also present in the myocardium of the left and right ventricles of the heart.

Histologically these areas were infarcts, areas of acute ischaemic necrosis resulting from thrombosis of afferent arterioles and capillaries. Small infarcts were also present in the red pulp of the spleen. Fibrin microthrombi were detected in arterioles and capillaries in PTAH stained sections of lung, heart and kidney. The widespread formation of microthrombi in small blood vessels which resulted in numerous infarcts in major organs is consistent with disseminated intravascular coagulation (DIC). PTAH positive thrombi were present in the lung of the other severely affected sheep, but no associated lesions were present in the lung or other organs. All four treated sheep suffered considerable loss of tissue fluid from the skin lesion; this was reflected in significantly low levels of serum albumin (2.0–3.5 g/dl) detected just before autopsy.

DISCUSSION

The bacterial counts on the wool and skin from the area of myiasis increased dramatically within 3 days of implantation and a variety of organisms were cultured. The effect of this bacterial flora on the area of damaged skin and the absorption of any toxins which may have been produced could have been instrumental in initiating toxaemia. On the other hand the absorption of toxic excretory/secretory products of the Lucilia larvae may well have contributed to the toxaemic state.

Pseudomonas aeruginosa was present in increasing numbers in the area of myiasis. In absolute terms this organism increased approximately 10^4fold during the progression of strike. However, total bacterial numbers increased 10^6fold and the low initial population density of Pseudomonas meant that this organism was never identified in culture plates at an incidence in excess of approximately one colony in every 1000 observed. Its role in the progression of myiasis and debility in the animals is therefore open to conjecture. Whereas Pseudomonas may

participate in attracting the sheep blowfly (Merritt and Watts 1978), the present studies do not incriminate it as a major factor in the pathogenesis of myiasis. It should be pointed out that the experimental sheep were artificially challenged and therefore showed no evidence of fleece rot prior to challenge. Our field studies will examine this issue further in an attempt to resolve this equivocal situation.

In one of the four sheep there was evidence prior to death of haemostatic defects, indicating the formation of intravascular thrombi. This was confirmed at autopsy when changes consistent with disseminated intravascular coagulation (DIC) were revealed. DIC is a non-specific process that may contribute substantially to the death of an animal (Thomson 1978), and is defined as the pathological activation of the coagulation mechanisms leading to intravascular clotting in arterioles and capillaries. It occurs in many conditions including endotoxaemia, shock and severe tissue damage (Thomson 1978). The occurrence of DIC in sheep artificially struck with Lucilia cuprina suggests that it is involved in the pathophysiology of sheep deaths due to blowfly strike.

SUMMARY AND CONCLUSION

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This contract describes a study aimed at revealing the sequence of events which occur in the sheep during severe myiasis. The pathogenesis of larval challenge embraced vast changes in the indices of cardinal physiology, haematology, bacteriology and pathology.

Merino wethers were subjected to daily implants of 500 Lucilia cuprina larvae for 8 days. Others were maintained as controls. The most significant physiological effects on infested sheep were elevated body temperatures and respiratory and pulse rates, complete loss of appetite and a sudden liveweight loss. The febrile response was marked; most animals demonstrated rectal temperatures well in excess of 40°C. The timing of this 'response was parallel with the extent of larval challenge, temperatures subsiding towards normal as larvae progressively left the sheep.

The marked haematological changes in the infested sheep were evidence of a severe toxaemic challenge. In two of these sheep a transient neutrophilia and an ensuing marked neutropenia with accompanying toxic degenerative changes were indicative of the fulminating nature of the condition. The other two sheep responded to challenge by summoning and maintaining a neutrophilia which in turn was associated with their recovery. Haemostatic defects which were in evidence in one animal prior to death were confirmed at autopsy which showed thrombi in small vessels and multiple infarcts in the kidney and heart. All these changes are consistent with severe toxaemia.

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