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The Effect of Anti-inflammatory Agents on the Clinical Expression of Bovine Ephemeral Fever

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ABSTRACT

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The effect of two anti-inflammatory drugs on the development and persistence of clinical signs in cattle experimentally infected with bovine ephemeral fever (BEF) virus was investigated by their administration, either before or after the commencement of fever. A total of 16 cattle was given phenylbutazone sodium (PBZ). The drug prevented fever and other clinical signs in six cattle when given daily during the incubation period, and at 8-h intervals for 5 days when clinical disease might be expected. When treatment with PBZ was deferred until 2–4 h after the commencement of fever, the rectal temperature returned to normal within 4 h in four of six cattle and the development of other clinical signs was suppressed. Clinical signs of ephemeral fever occurred in four untreated cattle infected at the same time. Viraemia, the development of neutralizing antibodies (at 8–11 days), resistance to subsequent challenge with BEF virus, neutrophilia, lymphopenia and a rise in plasma fibrinogen occurred in all BEF-infected animals whether treated or untreated, despite different clinical appearances. The mean peak of plasma fibrinogen in the untreated cattle was 6.9 g l^{-1}; 3.2 g l^{-1} when treated 2–4 h after fever developed and 3.8 g l^{-1} when treated from 18-h post-infection. BEF virus was isolated from leucocytes of each of the cattle, but the frequency of isolation was lower in the treated group. The results indicate that treatment with PBZ blocked the host response which produces the clinical signs and did not have an anti-viral effect. In a similar experiment, a long-acting anti-inflammatory drug, flunixin meglumine, failed to prevent BEF or to modify the clinical signs once they had developed, except for the rectal temperature which returned to normal within 2–4 h of the administration of the drug. The efficacy of this drug was not improved by increasing the dosage to two or three times the recommended level.

INTRODUCTION

Ephemeral fever is caused by an arthropod-borne rhabdovirus, bovine ephemeral fever (BEF) virus. The disease is of short duration (3 days) and is characterized by fever, ocular–nasal discharge, polyarthritis and a pronounced neutrophilia (Mackerras et al., 1940). Recent studies (St. George et al., 1988)

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have demonstrated that the fever may present as uni-, bi- or polyphasic. In severe cases, the animal is paralysed and unable to control limb movement and may be in sternal recumbency. Recovery usually begins when the fever subsides.

The pathogenesis of ephemeral fever is thought to be a consequence of vascular inflammation. There has been anecdotal evidence (St. George et al., 1984) that an anti-inflammatory agent, phenylbutazone sodium (PBZ), is beneficial in the treatment of ephemeral fever. Both PBZ and a recently introduced anti-inflammatory agent, flunixin meglumine (Finadyne, Schering Corporation, U.S.A.), interfere with the cyclooxygenase pathway and prevent the excessive production of prostaglandins (Lees et al., 1987). In the present study, cattle were experimentally infected with BEF virus and the effect of short-acting (PBZ) and long-acting (flunixin) anti-inflammatory agents on the course of the disease was investigated.

MATERIALS AND METHODS

Cattle

Cross-bred cattle of 8–18 months old were held in insect-proof accommodation and handled for 2–3 weeks before inoculation. All cattle were free of serum-neutralizing antibodies to BEF virus.

Viruses

BEF virus stock was a heparinized whole-blood suspension obtained from a cow naturally infected with ephemeral fever (Doherty et al., 1969). The isolate had been passaged 11 times in cattle and had a titre of $10^{5.1}$ tissue culture 50% infective doses per ml ($TCID_{50}$ ml^{-1}) in BHK21 cells. The virus strain used for serology was obtained from the same source but had been passaged six times in mice and 14 times in baby hamster kidney cells after a single bovine passage.

Experiment design

The investigation consisted of four experiments. The cattle within each experiment were divided into three groups and all the cattle within each group were inoculated intravenously with 2 ml of a pooled suspension of BEF-infected blood. All cattle were challenged 23 days after primary inoculation with a similar inoculum.

Experiment 1

Group A ($n=4$): nil treatment.

Group B ($n=4$): phenylbutazone (Nabudone injection, Ilium Products) treatment (8 mg kg^{-1}IM) was begun 2 h after the first animal in this group

showed a 1 °C rise in rectal temperature. These cattle were treated at 8-h intervals for 40 h after the first treatment.

Group C ($n=2$): PBZ treatment (8 mg kg^{-1} IM) 8 hourly for 80 h, commencing 44 h post-inoculation (p.i.), i.e. before the appearance of clinical signs.

At the onset of fever, the clinical signs of all cattle were recorded every 2 h p.i. and blood samples were collected at 20, 44, 68 h p.i., then every 4 h for 4 days and then daily for 7 days.

Experiment 2

Group D ($n=4$): nil treatment.

Group E ($n=4$): cattle were treated with PBZ (8 mg kg^{-1}) 2 h after a temperature rise of 1 °C was detected in each animal and treatment was repeated every 8 h for 72 h.

Group F ($n=4$): all cattle were treated with PBZ at 18, 41 and 65 h p.i. and then every 8 h for a further 80 h.

Experiment 3

Group G ($n=4$): nil treatment.

Group H ($n=4$): cattle were injected intravenously with flunixin meglumine at 2.2 mg kg^{-1} body weight. Treatment began 2 h after a 1 °C temperature rise was observed in each animal.

Group I ($n=4$): Flunixin treatment was identical to Group H except that treatment began at 18 h p.i. and continued daily for 7 days.

Blood samples were collected at 18, 42, 66 h, at 8-h intervals for 96 h, and then daily for 7 days.

Experiment 4

Group J ($n=4$): Nil treatment.

Group K ($n=4$): Cattle were treated daily with 4.4 mg kg^{-1} flunixin meglumine, commencing 2–4 h after a 1 °C rise in temperature in each animal.

Group L ($n=4$): Cattle were treated daily with flunixin meglumine at 6.6 mg kg^{-1} body weight, commencing 2–4 h after a 1 °C rise in temperature in each animal.

Clinical observations and sampling procedures

During the first 48 h, the cattle were observed twice daily and then every 2 h. Clinical signs were recorded immediately before blood was collected. At each sampling, 60 ml of blood was collected from the jugular vein and divided as follows; 2.5 ml in ethylene diamine tetracetic acid disodium salt (EDTA) for total and differential leucocyte counts and 20 ml in EDTA for fibrinogen estimations and virus isolation. The remainder was allowed to clot and the serum assayed for neutralizing antibody levels to BEF virus.

Virus isolation

Blood samples were centrifuged at $1500 \times g$ for 10 min at 4 °C, washed once with sterile phosphate buffered saline and then recentrifuged. The leucocyte layer was removed and stored at −100 °C. The method of virus isolation for Experiment 1 was as described by St. George (1986). Briefly, approximately 0.1 ml of the leucocyte fraction was inoculated onto *Aedes albopictus* C6/36 cells in 25-cm^2 flat plastic flasks and 1 week later subcultured into baby hamster kidney (BHK21) monolayers and observed for cytopathic effect. This method was unsuccessful and in Experiment 2 was modified by subculturing the *A. albopictus* cells, inoculated with leucocytes, onto Vero cells 7 days later and tested for the presence of BEF virus by immunofluorescence as described by Cybinski and Zakrzewski (1983). The method was further modified for Experiments 3 and 4. One ml of medium containing 10×10^6 *A. albopictus* cells was mixed with 0.1 ml of the leucocyte fraction for 1 h on a mechanical shaker. The cells were then resuspended in 4 ml of medium (2×10^6 ml^{-1}) and dispensed into 25-cm^2 flasks. After 48-h incubation at 26 °C the medium was removed and replaced with 5 ml of fresh medium. After incubation for 14 days at 26 °C, 0.1 ml of the *A. albopictus* cells was inoculated onto Vero cell monolayers and assayed for the presence of BEF virus by immunofluorescence after 30 h incubation at 37 °C. The medium used for the growth and maintenance of the *A. albopictus* cells was Leibovitz 15 medium (Flow Laboratories, Sydney) supplemented with 20% foetal calf serum, 5% tryptose phosphate broth, 600 μg ml^{-1} streptomycin sulphate (Commonwealth Serum Laboratories, Melbourne) and 75 units ml^{-1} Nystatin ('Mycostatin', Squibb, Melbourne).

Serology and haematology

Neutralizing antibody levels in the serum were estimated by the method described by Cybinski et al. (1978). All serum samples from an individual animal were titrated at one time to avoid test-to-test variation. In addition, each of the cattle was screened for antibodies to Kimberley virus (Cybinski and Zakrzewski, 1983).

Total leucocyte counts were made from the EDTA-treated blood using a Coulter counter (Model D2) and a smear of the same blood was fixed with methanol and stained with May-Grunwald Giemsa stain for leucocyte differentiation.

Plasma fibrinogen levels were determined by the clot weight method (Ingram, 1952).

RESULTS

Untreated cattle

The 16 untreated cattle described in Experiments 1–4 all developed clinical ephemeral fever. Three of the fever patterns are illustrated in Fig. 1. Leucopenia and a relative neutrophilia occurred in all 16 cattle. The duration of the neutrophilia varied from 24 to 72 h. The mean value of plasma fibrinogen in all 16 cattle before infection was 3.3 ± 0.7 (SD) $g\,l^{-1}$ and the mean peak value in clinically affected cattle was $7.95\ g\,l^{-1} \pm 2.3$ between Days 6 and 10.

The viraemia for the untreated group in Experiment 1 was not successfully measured. In Group D, it ranged from 20 to 72 h; Group G, 56–128 h and Group J, 14–102 h. The results cannot be statistically analysed as the sampling intervals differed and the technique of virus isolation was modified during the course of the study. BEF virus was isolated intermittently during the incubation period and most consistently at the sampling time just prior to and during fever. Virus was not detected after the rectal temperature had returned to normal. Neutralizing antibodies were first detected in all cattle between 8 and 11 days p.i. and titres rose gradually thereafter.

Phenylbutazone treatment

Experiment 1

Group B: PBZ treatment at fever. Fever developed in two of these cattle 93 and 102 h after infection. For several hours after the onset of fever, the differences in clinical appearance between the cattle in Groups A (control) and B (PBZ

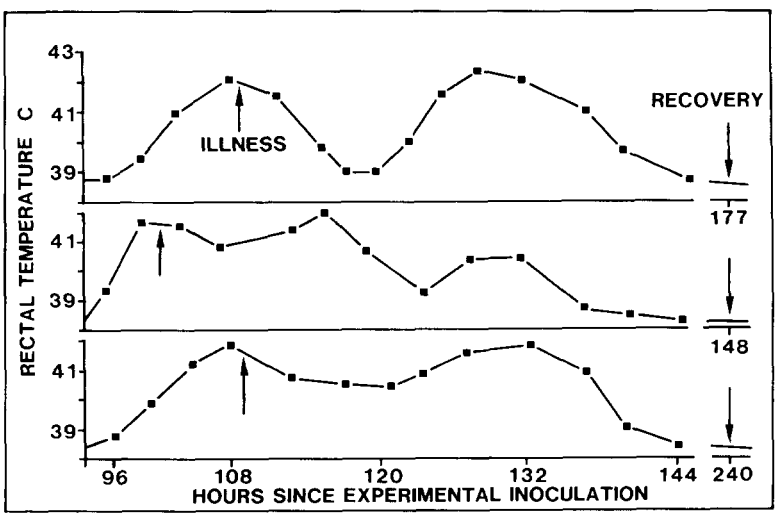

Fig. 1. Rectal temperature responses of three untreated cattle during infection with bovine ephemeral fever virus.

TABLE 1

Experiment 1: early and late treatment of ephemeral fever with phenylbutazone (PBZ)

Group	Treatment	No. in group	Fever	Other clinical signs	Leucopenia and neutrophilia	Neut antibody (day)	Immune to challenge
A	Nil	4	4	4	4	9–11	4
B	PBZ[1] at fever	4	2	2	4	8–10	2
C	PBZ from 44 h p.i.	2	0	0	2	8–12	2

[1]PBZ was administered to all cattle in the group 2 h after a temperature rise of 1 °C was observed in one steer.
[2]Anorexia, depression, nasal discharge, lameness, ruminal atony.

treatment) were not marked. The four animals in Group B behaved normally and the two febrile animals became afebrile from 4 to 16 h after treatment commenced. Two animals in Group A did not develop a fever or neutralizing antibodies until after challenge. The two afebrile cattle in Group B developed fever 9 h after the PBZ treatment was withdrawn.

Group C: PBZ treatment at 44 h p.i. Fever did not occur in these cattle during the period of observation and clinical signs of BEF were not observed. However, both cattle demonstrated changes in leucocyte numbers typical of the changes observed for BEF. The results are presented in Table 1.

Experiment 2

Group D: No treatment. All four cattle developed signs of moderately severe ephemeral fever commencing 65–101 h p.i. and finishing 22–96 h later. The temperature responses of three representative animals are shown in Fig. 1 and the mean temperature response of the four cattle is shown in Fig. 2. Since fever commenced at different times in different cattle, time zero is taken as the last observation before fever began so that a mean could be expressed.

Group E: PBZ treatment at fever. The first signs of fever in these four cattle began at 69–89 h p.i. They were treated intramuscularly with PBZ 2 h later. The fever subsided within 4 h and body temperature remained normal throughout the remaining period of intensive observation. No other clinical signs developed. The mean temperature adjusted to a similar zero point as described above for Group D is shown in Fig. 2.

Group F: PBZ treatment at 18 h. This group of four cattle remained healthy

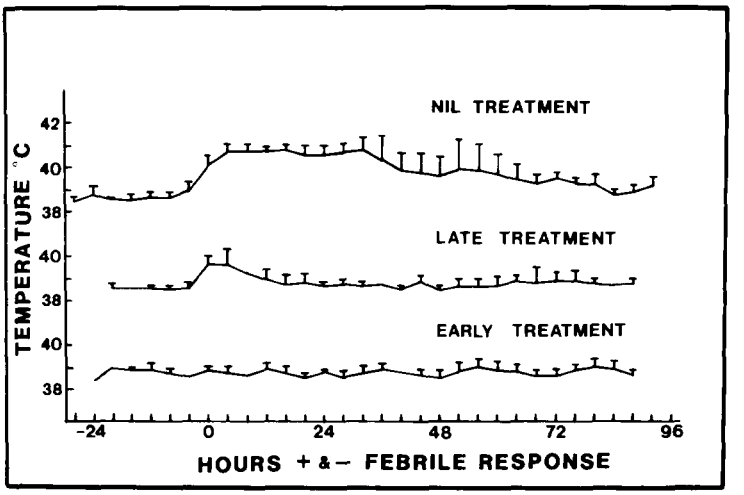

Fig. 2. Comparison of the temperature response of three groups of four cattle infected with BEF virus. One group was untreated, the second was treated with phenylbutazone (8 mg kg^{-1} body weight) from 18 h post infection and the third group was given phenylbutazone intramuscularly 2-4 h after fever commenced. Zero time is taken as 2 h before the first fever was detected. Bars represent 1 SD.

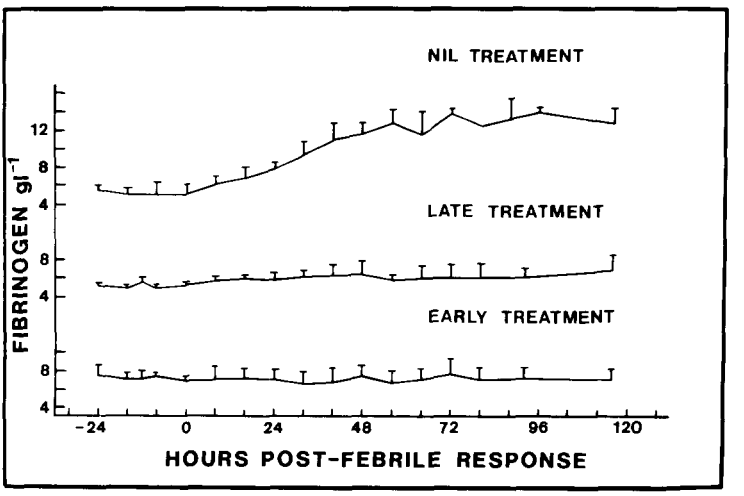

Fig. 3. Effect of phenylbutazone treatment (8 mg kg^{-1} body weight) on the fibrinogen response in the same cattle as described in Fig. 2. Bars represent 1 SD.

and afebrile throughout. The period between the first fever commencing in Group E and the last fever subsiding in Group D was taken for comparison and the mean of these observations collated (Figs. 2 and 3).

All the cattle in Groups D, E and F developed viraemias just prior to fever

TABLE 2

Experiment 2: early and late treatment of ephemeral fever with phenylbutazone (PBZ)

Group	Treatment	No. in group	Fever	Other clinical signs	Mean/max neutrophils	Mean/max fibrinogen ($g\,l^{-1}$)	Neut antibody (day)	Immune to challenge
D	Bil	4	4	4	15.2	6.9	9–10	4
E	PBZ[1] at fever	4	4	1	6.0	3.2	8–10	4
F	PBZ from 18 h p.i.	4	0	0	4.8	3.8	8–12	4

[1]PBZ was administered to an animal only after that animal showed a 1 °C rise in rectal temperature.

and neutralizing antibodies were first detected 9–12 days p.i. These cattle remained normal after subsequent challenge with BEF virus, in contrast to the susceptible animal challenged at the same time. A comparison of the clinical effects, haematological changes, mean temperature and fibrinogen responses of the three groups is shown in Table 2 and Figs. 2 and 3.

Flunixin meglumine

Experiment 3

Group G: Untreated. All four cattle exhibited typical clinical ephemeral fever with a triphasic temperature rise, commencing between 60 and 82 h and lasting 36–90 h.

Group H: Flunixin treatment at fever. All four cattle developed ephemeral fever, with fever commencing at 66, 68, 78 and 82 h p.i., respectively. The rectal temperature returned to normal 2–4 h after treatment then rose 10, 12, 14 and 26 h, respectively, after individual treatments. A slight clinical improvement was observed in 2 of the cattle but this did not correlate with the rapid fall in rectal temperature.

Group I: Flunixin treatment at 18 h p.i. All 4 cattle developed fever 76–160 h pi with 1, 1, 2 and 4-h rises, respectively, in rectal temperature of the individual cows. Between 2 and 4 h after injection of flunixin the rectal temperatures returned to normal. Clinical signs which developed following the first fever subsided in 2 of the 4 cattle after flunixin administration. Two cows underwent a second period of fever after further flunixin treatment. Results are presented in Table 3 as for Experiment 2.

BEF virus was isolated from all 12 cattle during fever and the late incubation

TABLE 3

Experiment 3: early and late treatment of ephemeral fever with flunixin meglumine (Finadyne) at 2.2 mg kg^{-1} body weight

Group	Treatment	No. in group	Fever	Other clinical signs	Leucopenia and neutrophilia	Mean/max fibrinogen ($g\,l^{-1}$)	Neut antibody (day)	Immune to challenge
G	Nil	4	4	4	4	6.1	10	4
H	Finadyne at fever	4	4	4	4	4.9	8–10	4
I	From 18 h p.i.	4	4	4	4	7.0	10–11	4

TABLE 4

Virus isolations from phenylbutazone or flunixin meglumine treated cattle and untreated cattle

Group[1]	Treatment	Range of viraemia (h)	Mean	No. isolations BEF virus[2]
D	Nil	28- 72	40	8, 11, 17, 19
E	PBZ at fever	4- 36	21	2, 7, 6, 8
F	PBZ early	4- 20	13	2, 2, 4, 5
G	Nil	57-156	109	8, 4, 11, 13
H	Flunixin at fever	72-202	124	13, 7, 8, 11
I	Flunixin early	69-133	88	7, 6, 8, 6

[1]No. = 4 for each group.
[2]Virus isolation techniques differed slightly for each experiment (see Materials and methods).

period and on the first day of convalescence, but not during the remainder of the sampling period (Table 4).

Experiment 4

Group J: Untreated. All four cattle exhibited typical ephemeral fever with two or four phases of fever, commencing between 72 and 122 h.

Group K: 4.4 mg kg^{-1} flunixin meglumine treatment at fever. All four cattle became febrile from 84 to 114 h p.i. Rectal temperatures returned to normal from 2 to 4 h after each dose of Finadyne but rose again 5–14 h later. Clinical disease in two of the cattle was mild. However, one of the remaining cattle developed severe respiratory distress and the other developed haematuria and died on Day 6 p.i. BEF virus was not isolated from the cow that died but was isolated from daily samples of the three remaining cattle 1, 2 and 4 days after clinical signs appeared. These three cattle resisted challenge after recovery.

TABLE 5

Experiment 4: treatment of ephemeral fever with high dosage (4.4 and 6.6 mg kg^{-1}) of flunixin meglumine

Group	Treatment (mg kg^{-1})	No. in group	Fever	Other clinical signs	Neutrophilia	Mean/max fibrinogen (g l^{-1})	Neut antibody (day)	Immune to challenge
J	Nil	4	4	4	4	10.6	6–9	4
K	4.4	4	4	4	4	7.3	6–9[1]	3[1]
L	6.6	4	4	4	4	6.1	6–9	4

[1] 1 cow died on Day 6 p.i.

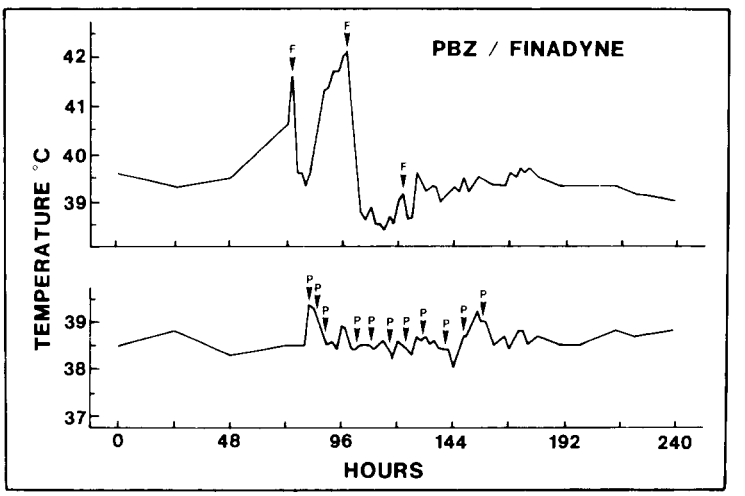

Fig. 4. Comparison of the response of the rectal temperature of ephemeral fever-infected cattle to treatment of the fever with phenylbutazone (8 mg kg^{-1} body weight) and flunixin meglumine (2.2 mg kg^{-1} body weight). P = injection of phenylbutazone; F = injection of finadyne.

Group L: 6.6 mg kg^{-1} flunixin meglumine treatment at fever. The four cattle developed fever from 63 to 88 h p.i. Two developed signs of mild ephemeral fever and two had moderate disease. Rectal temperatures returned to normal between 2 and 4 h after treatment but fever recurred 10–12 h after treatment. Two cattle developed haematuria. BEF virus was isolated from all four cattle for 1, 2, 2 and 4 days, respectively. The duration of viraemias varied from 1 to 5 days during fever and sporadically during the incubation period (Table 5). All animals resisted challenge.

The results of treatment with PBZ or flunixin at the onset of fever are compared in Fig. 4.

DISCUSSION

Recent observations have indicated that BEF is a non-cytolytic, inflammatory disease (Young and Spradbrow, 1980). The effect of 2 anti-inflammatory agents on the course of experimental disease was compared with that in untreated cattle. These control groups exhibited clinical signs of ephemeral fever and provided a basis for a subjective comparison with the treated groups. The biphasic and triphasic fever patterns recorded in these groups confirms earlier work by T.D. St. George et al. (unpublished data, 1988) who demonstrated similar patterns in experimentally infected cattle.

The techniques used to isolate BEF virus from infected cattle appeared to be superior to methods described earlier (St. George et al., 1984) and virus could be isolated during both the incubation and febrile periods. The intermittent appearance of virus during the incubation period may reflect the inability of the assay to detect virus at lower titres. Mackerras et al. (1940) used cattle inoculation as the assay method for BEF viraemia and found virus present during the incubation period and also on the third day of convalescence.

Prostaglandins have been shown to increase the synthesis of acute-phase plasma proteins by the liver (Kushner et al., 1981) and have been implicated as modulators of the primary antibody response (Goodwin and Webb, 1980). Administration of PBZ to ephemeral fever-affected cattle either modified or prevented the appearance of clinical signs of disease depending on the treatment regimen. If PBZ administration commenced on the day following experimental inoculation of BEF virus (Groups C and F), there was no expression of clinical disease. All six cattle remained free of clinical signs but developed neutralizing antibody. These results have been difficult to reconcile. In the past, the association between fever and infection has led to attempts to correlate host defence and immunity with hyperthermia (Duff and Durum, 1983; Hanson et al., 1983). The results presented here show that the development of immunity was not necessarily dependent upon a febrile response by the host. Moreover, changes to the leucocyte profile did not correlate with the development of immunity. This is consistent with the observations of others who have shown that the administration of cyclooxygenase inhibitors does not interfere with the normal leucocyte response (Scott et al., 1981). This was also apparent from the results of the experiment in which eight cattle were treated with PBZ at the onset of fever. Two of the cattle remained afebrile although a neutrophilia occurred in both these cattle and the remaining six febrile animals. This further demonstrates the complexity of the influence of inflammation upon the development of an immune response.

The treatment of infected cattle with flunixin meglumine was followed by an immediate and marked reduction in the rectal temperatures. This effect did not persist. Signs of toxicity, haematuria and respiratory distress occurred in cattle given higher doses in an effort to maintain the effect. The dose levels

were well in excess of those recommended by the manufacturer. It was concluded that the effect of flunixin was different from that of PBZ.

There is a striking similarity in the clinical expression of ephemeral fever to interferon toxicity in humans as described by Scott et al. (1981) and Scott (1982). High serum levels of interferon have been shown to occur in ephemeral fever (St. George et al., 1988). The administration of interleukin 1 (IL-1) is also known to result in fever and may also be involved in ephemeral fever. In addition, it has recently been shown that the release of IL-1 can be inhibited by anti-inflammatory agents (Bochner et al., 1987). Currently, controversy surrounds the relative roles played by IL-1 and interferon in the mechanism of inflammation and further work is required to study the influence of these lymphokines in the clinical expression of ephemeral fever. However, by specifically removing the ability to synthesize cyclooxygenase-dependent prostaglandins using PBZ and Finadyne, we have been able to modify the inflammatory response in vivo. Furthermore, interfering with the processes of inflammation produced no adverse effects on the host. This supports the view proposed by Uren and Murphy (1985) and St. George et al. (1988) that BEF virus is non-cytolytic and ephemeral fever disease is a result of either interferon or IL-1 activity.

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