

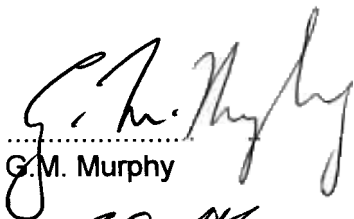
The biochemistry of ephemeral fever in cattle

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by

Murphy, G.M., St.George, T.D., Uren, M.F. and Collins, R.G.

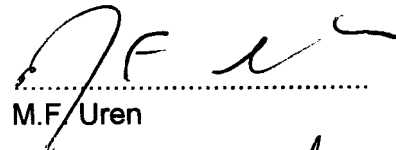
The study of the effects of infection with bovine ephemeral fever under field conditions has severe limitations. Accordingly, I arranged for cattle to be held in an air-conditioned room adjacent to the biochemistry laboratory so that certain of the tests could be done in real time. The prime purpose of the experiments reported here was the clinical chemistry. Dr St.George and Dr Uren provided veterinary supervision on a round-the-clock basis.


G.M. Murphy

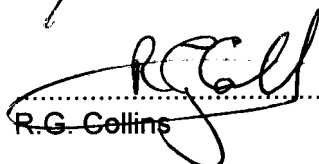
Date 30/5/2000


T.D. St. George

Date 30/5/2000


M.F. Uren

Date 18.7.00


R.G. Collins

Date 28/6/2000

THE BIOCHEMISTRY OF EPHEMERAL FEVER IN CATTLE

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Ephemeral fever is a disease of cattle caused by bovine ephemeral fever (BEF) virus and can be reproduced experimentally. Previously, we have made limited comment on the biochemical changes occurring during natural ephemeral fever infection (BEF) of cattle (S.George *et al* 1984; Uren and Murphy 1985).

A consistent observation was the marked drop in total serum calcium (Ca_T) levels on the day that clinical signs were most pronounced. Falls of more than $0.2 \text{ mM L}^{-1} Ca_T$ were common and frank hypocalcaemia ($Ca < 2.0 \text{ mM L}^{-1}$) occurred in an unpredictable percentage of affected animals.

Moseley and Axford (1973) considered stress induced hypocalcaemia in sheep in terms of increased lipolytic activity of adipose tissue. They demonstrated a highly significant, negative correlation between plasma Ca_T and non-esterified fatty acid (NEFA) levels. Prime, fat cattle are frequently noted to be worst affected during natural outbreaks of BEF (St. George *et al* 1986). In this paper we examine the role of adipose tissue as a Ca 'sink' during experimental infection, as well as the behaviour of ultrafiltrable calcium (Ca_u) during the clinical phase of ephemeral fever when viraemia is consistently present (St. George *et al* 1986).

A wide range of inflammatory conditions are known to evoke marked changes in circulating levels of zinc (Zn), copper (Cu) and iron (Fe) in mammals (Underwood 1977; Beisel 1977). Since cattle affected with BEF frequently exhibit neutrophilia and elevated fibrinogen levels, both consistent with an inflammatory event, we made detailed observations on the changes occurring in circulating Zn, Cu and Fe. Comparable observations were also made in animals treated with a specific cyclo-oxygenase inhibitor (phenylbutazone) (MF Uren, TD St. George and H Zakrzewski unpub data).

EXPERIMENTAL METHODS

Measurements were made on 2 groups of cattle: group 1 comprised 4 prime Hereford yearlings (mean live weight 275 kg). Cattle were fitted with novel bilateral jugular catheters (Takken 1984) several days prior to inoculation with BEF virus. Throughout the experiment, cattle were housed in a controlled temperature environment in individual metabolism crates. These were fitted with heavy rubber mats to prevent skeletal and muscle damage in recumbent animals. Intensive monitoring commenced 36 h after i.v. inoculation with known virulent BEF virus (strain M6). Vital signs (respiration, pulse, rumen function, reflexes) were manually recorded each hour for the next 5 days. Appropriate blood samples were taken at 2 hourly intervals throughout the same period. The analyses performed are listed in Table 1. Plasma ultrafiltrate preparations were commenced immediately after sample collection. Serum NEFA analyses were performed within 4 h of sample collection using a proprietary kit (WAKO Chemical Co., Japan). All cation analyses (Ca, Mg, Cu, Zn and Fe) were carried out by atomic absorption spectroscopy (AAS). Other metabolite and enzyme assays were performed by appropriate autoanalyser techniques (Gilford IMPACT 400). Haematological assays were carried out as described previously (Uren and Murphy 1985).

Group 2 consisted of 12 weaners assigned to 1 of 3 treatment groups: untreated controls; early treated; and late treated. Treated groups received i.m. phenylbutazone therapy (PBZ) either immediately after inoculation (early treated) or 6 h after the temperature rise (late treated). Dosage

TABLE 1

Biochemical and haematological measurements performed at 2 hourly intervals.

	<i>Unit</i>
Biochemical	
Calcium — Total (Ca_T) and Ultrafiltrable (Ca_u)*	mML ⁻¹
Magnesium — Total (Mg_T) and Ultrafiltrable (Mg_u)*	mML ⁻¹
Zinc	μ gL ⁻¹
Copper	μ gL ⁻¹
Iron*	μ gL ⁻¹
Inorganic phosphate	mML ⁻¹
Protein — Total and Albumin	gL ⁻¹
NEFA* (non-esterified fatty acid)	μ mL ⁻¹
Alkaline Phosphatase	UL ⁻¹
Creatine Kinase	UL ⁻¹
Haematological	
WCC — Total and Differential	$\times 10^9$ L ⁻¹

* = Measurements commenced within 2 h of the start of fever, other measurements commenced 36h after inoculation.

and frequency were adequate to maintain rectal temperature below 39°C. Throughout the experimental period, these cattle remained loose in an isolation unit. Blood was obtained by venipuncture. The protocols for physical and physiological monitoring were similar to, but less intensive than, those for group 1 animals. Labour constraints prevented the following measurements: plasma Ca_u and Mg_u and serum NEFA.

RESULTS

The individual reaction patterns for selected parameters of all animals in group 1 are illustrated in Figure 1. All measurements are adjusted to the onset of fever ($t = 0$) as the incubation period varied from animal to animal. The animals are numbered 1 to 4 commencing with the upper graph in each subset. Significant differences are readily apparent. Animals 2 and 3 show a single fever reaction of less than 24 h duration (Fig. 1c). Neither animal showed appreciable neutrophil response. Conversely animals 1 and 4 show a triphasic fever reaction of up to 72 h duration with marked bursts of neutrophilia.

Prolonged periods of hyperventilation ($RR \approx 40$ min⁻¹) were features of numbers 1 and 4; maximal rates were in excess of 100 min⁻¹. Probably the most visible difference between animals however, was that of sternal recumbency with an inability to rise. This was not seen in numbers 2 and 3, whereas both 1 and 4 showed this syndrome. The condition was apparent in both animals within 12 h of the initial temperature rise. Animal 1 recovered some 40 h after the initial episode; animal 4 failed to recover, lapsed into full lateral recumbency (44 h), and was euthanased 4 days later.

Histopathological examination of animal 1 showed very severe axonal swelling and Wallerian degeneration in the white matter of the anterior cervical cord. All funiculi were affected.

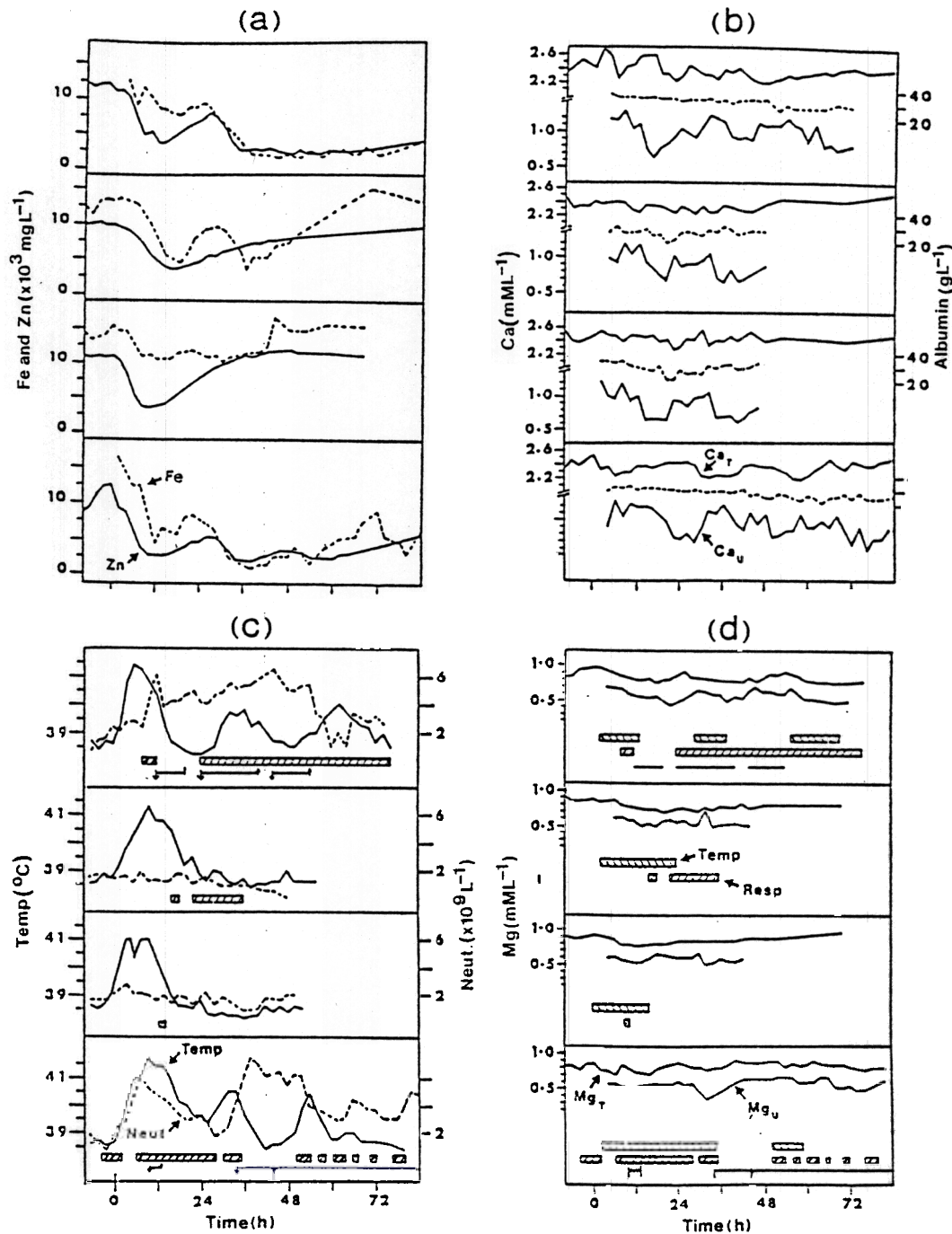


Figure 1. Cattle infected with ephemeral fever virus: changes after the onset of fever ($t = 0$) in (a) plasma iron (Fe) and zinc (Zn); (b) serum albumin, and total serum and ultrafiltrable plasma calcium (Ca_T , Ca_U); (c) rectal temperature, circulating neutrophils and hyperventilation (respiration rate $\approx 40 \text{ min}^{-1}$); and (d) total serum and ultrafiltrable plasma magnesium (Mg_T , Mg_U). Animals are numbered 1 to 4 commencing with the upper graph in each subset.

||||| indicates rectal temperature $\geq 39^{\circ}\text{C}$;

///// indicates respiration rate $\geq 40 \text{ min}^{-1}$.

ted with the ventral being worst. Areas of haemorrhage were present in the grey matter.

Clinical hypocalcaemia ($\text{Ca}_T < 2.0 \text{ mM L}^{-1}$) was not evident in any of the 4 animals. As indicated in Figure 1b plasma Ca_T fluctuated markedly ($\approx 0.2 \text{ mM L}^{-1}$) during the course of the viraemia; and fluctuations were most pronounced in animals 1 and 4. By comparison with

Ca_T , the changes in Ca_U are even more dramatic. In relative terms, Ca_U frequently fell to less than 35% of Ca_T (normal range $\approx 50\%$). Relevant data for plasma Mg (Mg_T and Mg_U) are shown in Figure 1d. There is little discernible evidence of Ca related Mg compensation.

Serum NEFA levels were significantly elevated in all animals throughout the viraemia.

Levels in all animals rose from $< 50 \mu\text{M L}^{-1}$ (pre-experimental) to lie within a range of 400 to 600 $\mu\text{M L}^{-1}$ during viraemia.

Serum albumin levels are depicted in Figure 1b. Again there are obvious differences between animals according to the severity of the viraemia. Whereas animals 2 and 3 (least affected) show a frank, but short lived, hypoalbuminaemia within 20 h of the onset of fever, numbers 1 and 4 (severely affected) show little change in albumin levels until 40 to 44 h after the start of fever.

Plasma Zn and Fe fell in all animals (Fig. 1a). The extent of the fall and its duration mimic the severity of the clinical disease. Again animals 1 and 4 show the greatest response. Plasma Cu levels rose in all animals about 36 h after the start of the febrile response. Animals 2 and 3 showed little increase ($< 200 \mu\text{g L}^{-1}$) from the initial mean level of $980 \pm 30 \mu\text{g L}^{-1}$, whereas significant increases ($\approx 600 \mu\text{g L}^{-1}$) were found in numbers 1 and 4.

Plasma alkaline phosphatase (AP) and creatine kinase (CK) activities both reflect the severity of clinical signs. AP showed little change in animals 2 and 3 but fell to less than 30% of initial activities (66 and 45 UL^{-1} in numbers 1 and 4 respectively. CK activity remained below 50 UL^{-1} in numbers 2 and 3 throughout the course of viraemia but significant increases occurred in both numbers 1 and 4 (Fig. 2). In animal number 1, CK began to rise 52 h after the onset of fever and 4 h prior to it regaining its feet. CK rose from 32 UL^{-1} to 1 500 UL^{-1} within 10 h before falling to near normal (140 UL^{-1}), 86 h later. In animal 4 (the animal that had to be euthanased), CK

began to rise 40 h after the onset of fever and 4 h prior to its collapse into complete lateral recumbency. Values rose to $\approx 1\ 500 \text{UL}^{-1}$ within 24 h but then commenced to fall until, at euthanasia (100 h after initial rise), CK activity was $< 500 \text{UL}^{-1}$.

Feed and water intake of marginal reactors (numbers 2 and 3) showed little if any deviation from pre-experimental patterns. In contrast, severely affected animals (numbers 1 and 4) stopped drinking and eating at or about 30 h after the start of fever. A typical reaction pattern is shown in Figure 3. Cessation of food and water intake appeared to coincide with loss of the swallowing reflex. This was accompanied by a variety of signs of varying intensity and duration. These included: excessive lachrymation, nasal discharge, profuse drooling of saliva, bloat, ruminal stasis and recumbency.

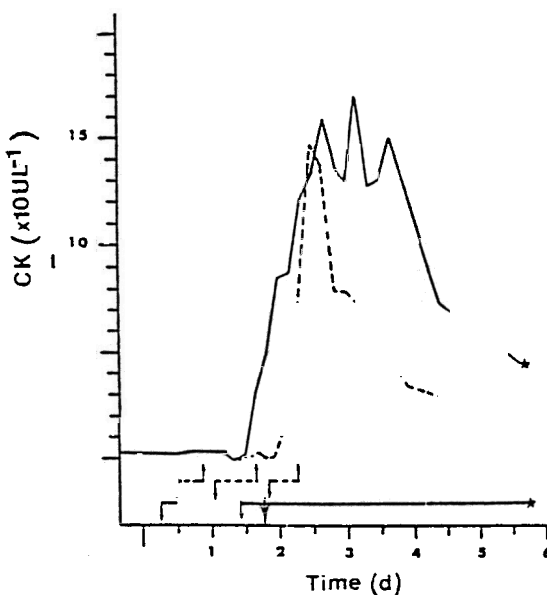


Figure 2. Cattle infected with ephemeral fever virus: changes after the onset of fever ($t = 0$) in Creatine Kinase activity of two severe reactors: number 1 (---), and number 4 (—) (hr1).
 ↓ (---) ↑ indicates period of sternal recumbency.
 —* indicates onset of lateral recumbency terminated by euthanasia.

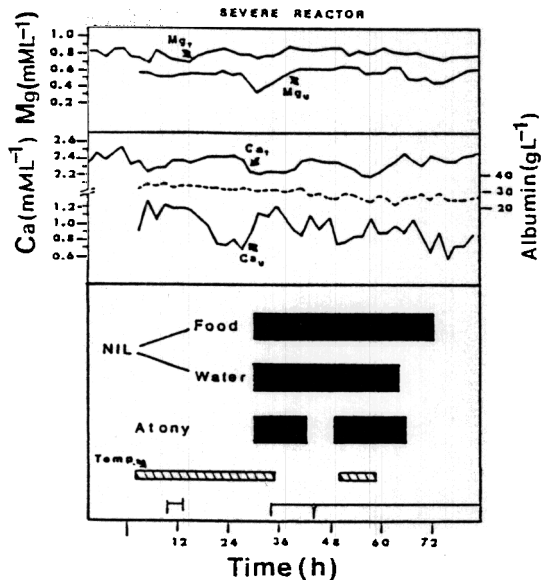


Figure 3. Cattle infected with ephemeral fever virus: typical reaction pattern of a severely affected animal (number 4).

Selected results for a typical reactor from each of the 3 treatments in group 2 (PBZ experiment) are portrayed in Figure 4. Fever was effectively controlled by PBZ treatment but a neutrophil response to viral challenge remained.

Plasma Ca_T fluctuated markedly during the 48 h following the onset of fever, irrespective of treatment (Fig. 4b). Minimum values of 2.06 and 2.07 mM L^{-1} are compared with pre-experimental levels of 2.57 and 2.55 mM L^{-1} for the early and late treated animals respectively. Frank, clinical hypocalcaemia (1.95 mM L^{-1}) was evident in the control animal at 48 h after the start of fever. No compensatory changes occurred in plasma Mg_T .

Significant decreases occurred in plasma Zn in all 3 animals (Fig. 4b). Maximum depression appears coincident with peak neutrophilia.

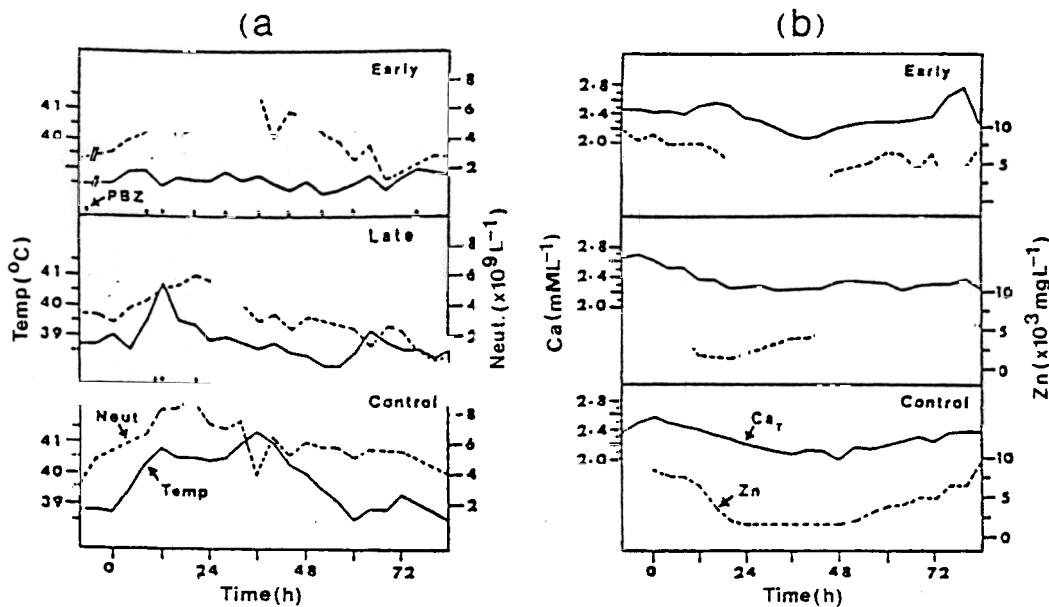


Figure 4. Phenylbutazone (PBZ) treatment of cattle infected with ephemeral fever virus: changes in (a) rectal temperature and circulating neutrophils and (b) total serum calcium (Ca_T) and zinc (Zn) in cattle left untreated (CONTROL), treated immediately after inoculation (EARLY), or treated 6 h after the onset of fever (LATE).

DISCUSSION

This experimental evidence confirms our earlier observations (St. George *et al* 1984; Uren and Murphy 1985) that transient falls of more than 0.25 mM L^{-1} in serum Ca_T are a significant feature of BEF affected cattle. Although classical hypocalcaemia ($Ca_T < 2.0 \text{ mM L}^{-1}$) was evident in only 1 animal during the viraemia (group 2: control), serum Ca_u levels fluctuated markedly in all animals monitored for this indice (group 1). The duration, frequency and magnitude of the falls in Ca_u paralleled the severity of the disease and were expressed clinically as tachycardia, muscle tremor, ruminal stasis, inability to swallow, and recumbency. This is consistent with the response to calcium therapy reported by St. George *et al* (1984): we are unaware of any similar studies demonstrating this facet of a viraemia.

The underlying mechanism(s) responsible for this aberrant calcium metabolism is not clear. Starvation and/or impaired digesta flow *per se* are not considered to be primary factors. Marked falls in plasma Ca_u and serum Ca_T occurred within 18 h of the onset of fever, whereas the severely reacting animals (group 1: numbers 1 and 4) continued to eat and drink for a further 12 h, at which time (30 h) ruminal stasis was first observed. Likewise, a hypoalbuminaemia related hypocalcaemia does not provide a satisfactory rationale for the behaviour of serum Ca_T . Transitory hypoalbuminaemia (albumin $< 25 \text{ g L}^{-1}$) did occur in mild reactors (group 1: numbers 2 and 3) at or about 24 h after the temperature began to rise. However, it did not occur in severe reactors (group 1: numbers 1 and 4) for a further 36 h. At this time, serum Ca_T had begun to rise

although Ca_u continued to fluctuate significantly, in both absolute and relative terms.

The experimental data from group 1 does suggest 2 factors which could account for at least part of the behaviour shown by Ca_T and Ca_u during the viraemia. Firstly, prolonged hyperpnoea increases blood pH and promotes formation of the albumin/calcium complex at the expense of ionizable calcium pool. Respiratory distress did occur in all animals; and the degree and duration closely followed the severity of the clinical signs (Fig. 1c). Thus fever related hyperventilation with attendant alkalemia could account for the fluctuations in Ca_u . However, in the absence of blood gas/pH measurements, this proposal remains conjectural. Secondly, increased lipolytic activity of adipose tissue is accompanied by a significant rise in plasma free fatty acids (Akgun and Rudman 1969). In sheep subjected to a variety of stressful stimuli (minor surgery, restrictive handling and adrenalin infusion), Moseley and Axford (1973) demonstrated a 3-fold increase in plasma NEFA levels ($\approx 500 \mu\text{M L}^{-1}$). This was accompanied by a 0.25 mM L^{-1} drop in serum Ca_T . *In vitro* studies with adipose tissue supported their hypothesis 'that under the effect of stressful conditions, calcium is transferred from plasma into adipose tissue and this transference of calcium may be proportional to the degree and duration of stress'. We found plasma NEFA concentrations increased in all animals during the viraemia. The greatest increases ($\approx 550 \mu\text{M L}^{-1}$) occurred in the worst affected.

Infection, bacterial products such as endotoxins, and many inflammatory agents, increase plasma Cu (Underwood 1977) and decrease plasma Zn and Fe levels (Beisel 1977).

These changes are considered beneficial responses, since Fe availability determines the growth rate of many bacteria (Bullen 1981), and Zn is known to inhibit phagocytosis (Sugarman 1983). The increase in Cu reflects *de novo* synthesis of ceruloplasmin. This is proposed to have an essential role as an aqueous phase scavenger of free oxygen radicals produced by phagocytic leukocytes (Clark *et al* 1985). Current opinion identifies these trace metal changes as part of a reaction cascade mediated via Interleukin-1 (Dinarello 1984; Oppenheim *et al* 1986). Analogous behaviour of Zn, Fe and Cu show during BEF infection suggest that an Interleukin-1 cascade might be involved in the physiological expression of the disease.

Several aspects of the available experimental data support this possibility. The inflammatory nature of the disease is well documented (Mackerras *et al* 1940; Basson *et al* 1970; Young and Spradbrow 1980; Young and Chung 1986; St. George *et al* 1986). More importantly, the neutrophilia and trace element behaviour in PBZ treated cattle (group 2) show that these responses are independent of fever *per se*. This is in complete agreement with the results of Sobrado *et al* (1983). They demonstrated that suppression of fever via a comparable specific cyclooxygenase inhibitor (ibuprofen) did not affect whole body trace metal, haematological, or hepatic acute-phase-induced responses to leukocytic pyrogen or endotoxin. Likewise Scott *et al* (1983) found indomethacin prevented symptoms but did not suppress the neutrophilia and elevated corticosteroids or prevent the fall in plasma zinc seen in human volunteers given toxic levels of $\alpha 2$ interferon.

Clark *et al* (1985) have argued that tissue damage described as 'inflammation' should be considered as a consequence of the release of free radical species generated by invading phagocytic leukocytes. Interleukin-1 promotes extravascular infiltration of polymorphonuclear leukocytes (Oppenheim *et al* 1986; Lewis and Granger 1986) and this is a prominent feature in BEF affected cattle (Young and Spradbrow 1980; Young and Chung 1986). The clinically significant, though relatively short lived elevations in CK levels in severe reactors (group 1: numbers 1 and 4) are consistent with such an occurrence.

Basson *et al* (1970) found discrete, localised areas of muscle necrosis accompanied by neutrophil infiltration of the sarcoplasm in severe BEF reactors, killed at a late stage of disease. Unfortunately they did not measure the CK levels in these animals.

Other factors may have contributed to the elevated CK activities in this present study. The extensive lesions in the anterior cervical cord of the euthanased animal (group 1: number 4) are similar to those diagnosed as the cause of continuing ataxia and/or paralysis in refractory field cases of BEF (Hill and Schultz 1977). Grossly elevated CK activities ($\approx 20 \times 10^3$ UL⁻¹) are expected in such "downer" animals in which extensive pressure induced muscle damage can

occur in as little as 6 h (Cox *et al* 1982). However, even though animal number 4 was recumbent for 4 days, CK activity did not exceed 1.6×10^3 UL⁻¹ and in fact had fallen to < 500 UL⁻¹ at euthanasia. Likewise the behaviour of CK activity in the other severe reactor (group 1: number 1) is atypical of that normally seen in "downer" cattle (Cox *et al* 1982).

Overall, many of the clinical signs and biochemical changes occurring during BEF are analogous to those seen in toxaeemias with a chemical or bacterial origin. We have confirmed that, as is the case with staphylococcal toxic shock syndrome in humans (Chesney *et al* 1983), uncompensated hypocalcaemia is intimately connected with the clinical signs of the disease. The fact that the biochemical aberrations occurring during the viraemia are not dependent on fever has important implications for the short term management of infected cattle. Treatment with nonsteroidal anti-inflammatory drugs such as phenylbutazone, indomethacin (Scott *et al* 1983) and ibuprofen (Sobrado *et al* 1983) will produce afebrile, asymptomatic animals. Our results demonstrate quite clearly that despite "treatment", these animals remain biochemically dysfunctional.

Further studies are needed to determine if the biochemical sequelae of ephemeral fever involves an Interleukin-1 mediated cascade. Disturbed calcium homeostasis should also be investigated as an intrinsic component of the pathogenesis of other disease states characterised by an inflammatory response.

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DISCUSSION – SESSION 7B

Chairman: D Hoffmann

Ephemeral Fever Pathogenesis

Q Cox. How long after the onset of this injected dose of BEF virus did you post mortem that animal? *Q Young.* The animal with immunoperoxidase staining? *Q Cox.* No, an animal with muscle degeneration where oedema had spilled over from the muscle planes. *A Young.* Because there is a variable incubation period, I standardised the second day of clinical disease to autopsy the animals. The first day of clinical disease is one in which there is fever, the animal is off colour and looks sick. The second day is one in which lameness starts and muscle oedema appears.

Q Osburn. What do you consider as pathognomonic lesions of ephemeral fever. *Q Young.* You mean histological lesions? *Osburn.* There are many things which can lead to similar types of lesions as you have discussed. *A Young.* There is not a pathognomonic lesion of ephemeral fever. If an animal has fever, lameness and neutrophils in the joint fluids, then this is a good indication that the lesion of the disease is significant.

Q P. Doherty. I suspect that my colleagues in ANU would interpret this as a typical incident of free radical induced pathology. You would say that because there are lots of neutrophils, there are free oxygen radicals produced in the lesion sites which cause vascular permeability changes. You should look and find, for instance, products of lipid peroxidation. Have you looked at that aspect at all? *A Murphy.* The proposition refers to recent work that Ian Clarke, ANU, Canberra has done. They have reviewed the concept of the sort of biochemical changes that we are seeing here, of the inflammation process in a wide variety of models. These can, in fact, be considered in terms of increased peroxidation of free radicals (oxygen and hydroxyl radicals). These are known

to be detoxified to peroxide which is then broken down by peroxidase. This could be happening in phagocytic cells and could eventuate in all sorts of things eg. muscle damage. We have not looked at that yet. I do plan to, because we are also using this as a model to look at inflammation in other disease states.

Q P. Doherty. If I recall correctly, hydroxyurea was used as a free radical scavenger. *A Murphy.* Yes, well the interesting thing here is that it poses a possible explanation for the host response in dropping iron levels as well, because 1 of the positive factors of decreasing plasma iron during this acute reaction phase would be to slow down the autocatalysis of those radicals, or generation of those radicals.

Q P. Doherty. Considering these vascular permeability changes, did you look at CNS to see whether there was leakage into it? *A Young.* No. There were no vascular permeability changes in CNS, in either the brain or the spinal cord.

Q Woolcock. Bone marrow: are techniques sensitive enough, do you think, to detect low titre virus in the tissues? *A Young.* The technique that Dr Young Chung has developed (immunoperoxidase technique using monoclonals), certainly gave a much better result with the buffy coat preparations than we have ever seen before. We believe the technique that is becoming available is much more sensitive than previously, so we hope that we can detect small quantities of virus. Dr Young Chung also plans to do immunoelectron-microscopy of the samples he has collected, so that will give another guide as to where the virus is replicating. *Comment St. George.* I take it from that you are wondering whether the virus is persisting in bone marrow. Well even if it were,

in the recovered animal there is a sea of antibodies specifically against the homologous virus so its fate is swift if it multiplied and came into the circulation from the bone marrow. I do not think it would rise to a titre sufficient to infect a mosquito or a *Culicoides*.

Q Osburn. I am curious as to whether there was any evidence of proteinuria. Did you do any urine analyses? *A Murphy.* We did look but there was none. We have, in fact, done a full metabolic balance on these animals. Obviously it is very difficult in animals in full lateral recumbency. One of the possibilities we did look at in terms of the calcium loss was the possibility of oligouric or nonoligouric renal failure, but we did not find that. But again, that has been 12 animals so far, so it is not exhaustive, but it is an exhausting process.

Q Woolcock. The Wallerian degeneration of cervical cord – firstly to Peter Young, is that the only neurological lesion that has been described in ephemeral fever? And secondly, is it proposed that this has a toxic cause? *A Young.* The answer is yes, and until this particular case, reported here today, the only time it had been seen was in field cases. Mike Hill's series is the only report we know of. *A Hill.* We had no trouble demonstrating this lesion but we do have a lot of trouble trying to explain its cause. It is bilaterally symmetrical in the ventral ventriculi of the spinal cord, so it is most unlikely to be toxic. Our theory was that it was produced traumatically and it was virtually always in the anterior cervical cord, usually in C1. The theory we put forward was that for some reason when the animal fell ill with ephemeral fever, it suffered very severe ventroflexion and this put pressure on the cord and the lesion developed then. The trouble with this theory was that Gerry Murphy was observing his animals very closely and this was not observed to happen so we are in a vacuum at the moment. We are not sure why this lesion develops. Trauma would still probably be the most likely cause, presumably very similar to dogs with a disc herniation.

Q Hoffmann. The second part of Brian's question, was the theory that it is a toxic syndrome you are looking at. *A St. George.* There are well-known plant toxins that produce peculiar lesions in the spine, but I do not think Wallerian degeneration fits into that category does it Mike? *A Hill.* No, this is a focal lesion and bilaterally symmetrical. I believe it is unlikely to be produced by a toxin e.g. humpy-back of sheep. *A St. George.* I have no explanation.

Q Francis. Paraplegia, of course, is one of the main clinical signs and the main cause of a loss in ephemeral fever. The point we have been mentioning is the cause of the paraplegia. Do you know the cause of the paraplegia? *St. George.* I think Mike Hill examined animals because they were paraplegic or quadraplegic. He was the first

one to look high enough up the spinal cord and find the actual lesion.

Q P. Doherty. I am interested in the animals treated with calcium borogluconate. Do they always get up? If they always get up then it is not due to some sort of nerve damage but due to something to do with calcium imbalance. *A St. George.* Seven out of 9 treated cases responded completely although 1 was only very staggery. One changed from being completely comatose, with reflexes gone and with Cheyne-Stokes breathing, to fully alert in 11 minutes, drinking several gallons of water and attempting to get up. It did not have full control of its hind limbs, but it was a fairly advanced case. The other comatose cow got to the stage of sitting up completely and had control of itself. It attempted to get up, and ate and drank normally, with heart rate back to normal. However, both of these cases relapsed. I had only given them 1 treatment because at that stage I was so impressed with my results that I did not think they needed to be followed up.

Q McManus. I do not think you can eliminate the possibility of exertion rhabdomyelitis being involved here. It is a regular phenomenon where you have a hypocalcaemic situation. You can partially reverse it by injecting calcium, but can not replace muscle damage. I would like to ask too, whether you looked closely at the CK levels. In Tasmania, we have done some rather unusual work on, of all things, stranded whales, and we feel that the CK levels are an absolute indication of stress. The level shoots up rapidly in an animal that is in a very stressful situation and it is unlikely to reverse from that because of the stress of the actual event. Did you look at that more closely? *A Murphy.* This is in terms of what electrophoretic separation isofocusing, or the enzymes themselves? *Q McManus.* Enzymes themselves? *A Murphy.* Doing isozyme format of it? *McManus.* Yes. *Murphy.* No, we did not. The problem was that those values I am showing you were taken every 2 h for 7 days. We were trying to do analyses in real time and unfortunately the pressure of work prevented it. The problem I have in reconciling the CK values with both the Wallerian degeneration that Mike Hill reported in his field studies and the typical downer syndrome in cattle is that these values represent probably only 1/20 or 1/30 of what one would normally expect to see. If one goes back to work that we have published on transport stress of cattle coming, say, from Townsville, north Queensland 1800 km away to Brisbane, downer animals in that situation which show classical hypocalcaemia and glucosuria would have CK levels of the order 50–60,000. The values in ephemeral fever were of the order 1500–1800. This is our problem – they are not really high enough to be associated with what one would expect to see in a severe cervical lesion and also the levels did fall even while the animal remained totally recumbent. With muscle damage, you would expect to see them continuing to go up.

Q Francis. Would some of them, especially those that were thrashing around, be more stressed? *A Murphy.* Yes.

St. George. We do not have any controls. I do not think C1 is looked at in downer milk fever cases? *A Hill.* Only in extreme circumstances. I think the point that ought to be made here is that a cervical lesion is a very unusual sequela to ephemeral fever. We are not proposing that this Wallerian degeneration of the spinal cord and lesions are usually responsible for animals going down with ephemeral fever.

Q Spradbrow. The component of this interaction which we know so little about is the virus. We have a parasite that is not obviously killing cells in the cattle host. It certainly is not killing any of the cultured bovine cells where we have been looking for it. Do we know where it is going after the infection? What organs is it in? What is it doing there? *A Young.* No. I do not think we know much more now than we did 5 years ago. Dr Young Chung has in progress some experiments which may give the answer. The spot that

we are thinking about more and more now is bone marrow. We have seen the virus in neutrophils, both in fluids, in body cavities and the blood. We do not propose that the virus is actually replicating in the neutrophils, because I do not think that neutrophils have the wherewithal to support viral replication. It is possible that replication does occur in some stem cell and the virus ends up being carried around the body in neutrophils. Certainly this is 1 of the great unknowns of BEF. Where does the virus replicate?

Q Osburn. What types of interferon were assayed for: was it total interferon? *A St. George.* Total interferon, and I must stress that I could not control the tests with synthetic interferon because there was none available in Australia at the time. Up to the present I am unable to get hold of bovine interferon, although Genetech has offered some. We did characterise the interferon and it appears to be partly heat labile and partly pH labile. These criteria of classifying interferons seem to be going by the board. We appear to have a mixed bag of interferons.